Table of Contents

Class Policy for BOT 3015L

Plant biology lab is an upper-division elective course that fulfills one of the five required laboratory courses required for a major in biological science. Plant biology lab is designed to correlate with and reinforce the concepts of the plant biology lecture (required for a major in biological science). Experiment design and basic statistical data analysis are important aspects of the lab course. The objectives of the lab course focus on structure and function relationships, anatomy, physiology, symbioses between plants and organisms of other kingdoms, reproduction, and evolution. In addition to the plant kingdom, the lab introduces the fungi and protists.

Prerequisites

Before a student registers for 3000- or 4000-level courses in the Biological Sciences, he or she must have satisfactorily completed (C- or better) the following courses or equivalent: BSC2010/2010L, BSC2011/2011L, CHM1045C and CHM1046C. In addition, BOT 3015 is a co- or prerequisite for BOT 3015L. The information and experiences in these courses will be built upon and referred to in this course.

Insurance

Students must have accident insurance. FSU does not accept responsibility for accidents, even if the accident occurs when the student is engaged in an FSU-sponsored activity (such as this laboratory) and even if the student is not negligent. Instructors will not aid in cost recovery for any accidents that may occur while attending this course.

Required materials

- Access to a personal computer with Microsoft Excel and printer (undergraduates may access computers in the Conradi Computer Laboratory, and many computer labs around campus exist)
- Laboratory notebook with unlined paper (bound, three-ring binder, or folder with clasps in the middle)
- Textbook: Biology of Plants by Raven *et al.* 7th edition brought to class each week
- Laboratory manual (Target packet)

Attendance

Attendance on the first day of class is required university wide; Biological Science will administratively drop those not present. Student attendance will be recorded via student signature at the beginning of each class and verified by the instructor. Students arriving more than 15 minutes late will be marked absent. A student may miss only one lab period during the semester (with or without excuse) without penalty beyond missed graded work. For each additional lab period that is missed, *a penalty of one letter grade from the final course grade for each absence will be applied.* Documentation does not excuse a student from missed lab periods in excess of the initial absence and thus, the penalty will still be applied. Make-up labs will not be given; however, prior (at least 48 hours) arrangements with TAs to attend another lab section will be considered. If a lab period is missed during which a quiz is given or on which a report is based, the student may make-up the quiz or experiments if proper documentation of a valid reason is presented at first opportunity (within 48 hours of documented event) after the missed period. All documentation will be subject to verification. Falsified documentation will be handled according to the Academic Honor Policy (see below). Late assignments and delay in make-up will result in grade penalty (20% per day) for the quiz or assignment. Valid documentation includes:

- sickness: a dated certificate of illness signed by a physician (not within immediate family) with a verifiable address and telephone
- legal: statement from a court confirming the student's presence in court proceedings
- other legal document confirming an emergency that prevented the student from attending class

Students may be excused for religious holidays if notification is given at least 24 hours prior to the beginning of class.
Students are responsible for all material covered during any absences.

ADA statement
During the first week of class, students with disabilities needing academic accommodation should (a) register with the Student Disability Resource Center and (b) present a letter to the instructor from the Center indicating the need that is to be accommodated. Then, the student and instructor will make a written contract that indicates how instruction and testing will deviate from that of the general student population.

Academic Honor Policy
All students are required to uphold the Academic Honor Policy (AHP) that is found in the FSU *Student Handbook* and at http://www.fsu.edu/%7Edof/forms/honorpolicy.pdf at all times. Violations of the AHP include (1) plagiarism (presenting (*e.g.* written, oral, graphical) without acknowledgement of source (*e.g.* web, print, lab manual, work of other students)), (2) cheating (*e.g.* copying work of other students (past and present), unauthorized use of notes or devices (*e.g.* cell phones, calculators), discussion of examination contents before scheduled examination)), (3) unauthorized group work (group activities in this course include discussion and data collection, but *all* writing must be completed individually), (4) fabrication (*e.g.* making up data or not reporting knowledge of falsified data, creating laboratory notebook drawings from textbook figures), (5) falsification (*e.g.* altering data), (6) misrepresentation (*e.g.* signature imitation, false excuses), and (7) submission of same or similar work as previously credited work. All violations will be treated as explained in the AHP.

Performance and Participation
Students are required to read the lab materials before each class and are expected to participate actively in all laboratory activities. Prior to each class, each student is responsible for preparing a drawing list (minimally title and magnification(s)) based on the directions in the lab manual, independently completing (in complete sentences) the review questions at the end of each chapter, and writing step-by-step protocols when applicable.

Safety
It is our goal to maintain a safe learning environment for all students. No food or drink is permitted in the laboratory. Instructions given by the instructor must be followed or the student will be asked to leave the laboratory and will be counted absent for the laboratory. Disrespect for instructors or fellow students will not be tolerated and will also result in a student being asked to leave the laboratory. All students are required to uphold the Honor Code, found in the FSU *Student Handbook*, at all times.

Grading Policy

Grading scale: A ≥ 93 > A- ≥ 90 > B+ ≥ 87 > B ≥ 83 > B- ≥ 80 > C+ ≥ 77 > C ≥ 73 > C- ≥ 70…

Course grade breakdown
Three quizzes (total 30%) will be administered in the first 30 minutes of indicated lab periods. Each quiz will cover material from previous lab periods. Quizzes will have short-answer, multiple choice, and practical-style questions (drawings may be required). The objectives at the beginning of each topic provide a guide. During quizzes, no contact with cell phones, PDAs, calculators, chewing gum wrappers, or any other potential cheating materials is permitted. Violations and intention of violations of the AHP that involve quizzes minimally result in loss of credit for the quiz and penalty of one letter grade for the course and maximally result in an F for the course.
The final quiz (20%) will be cumulative and in similar format to the other three quizzes.

Two short experiments (10% each) will be independently written (see the guide to organizing a short experiment report, Chapter 1). Grades are based on understanding of scientific concepts and the scientific method. Violations and intention of violations of the AHP that involve experiments and lab reports minimally result in loss of credit for the experiment and report and penalty of one letter grade for the course and maximally result in an F for the course. Electronic (via www.turnitin.com) and paper copies must be submitted.

The full experiment report (15%) will be a more extensive report than the other two reports (as the experiment is more extensive). Although there will be group participation in gathering the data, each student is responsible for an independently written report (see Chapter 1 for details). Grades are based on understanding the background of the experiment and the design of the experiment, interpreting the results of the experiment utilizing statistical tests, and effort in conducting the experiment. Electronic (via www.turnitin.com) and paper copies must be submitted.

The lab notebook (15%) will be randomly collected and checked for completion and detail. See the introduction in this manual (Chapter 1) for details on maintaining a lab notebook.

Appeals
Appeals—based on truly extenuating circumstances—for exemption to this policy should be to Dr. Outlaw (outlaw@southernmatters.com, BIO 306)

Chapter 1

Orientation to Plant-Biology Lab

Why study plants? An introduction to plants

Plants sustain life on earth by trapping light energy and converting it into stable chemical energy. Photosynthetic organisms appeared about 3.5 billion years ago (the first human-like primates appeared a mere 2 million years ago)[1]. Plants have evolved into biochemically complex organisms that dominate the landscape.

Human civilization has long depended on plant life for sustenance. We have exploited plant resources in several ways, primarily for food and fiber through agriculture and for energy through fossil deposits. In order to utilize plant resources, we must gain a better understanding of how plants work. This understanding began at the dawn of civilization, and today we are in the midst of an exciting era in plant biology.

Scientists in fields ranging from taxonomy to biochemistry and molecular biology are elucidating the diversity and complexity of plant life. In the last several decades, we have gained a significant understanding of various plant processes. Our basic understanding has served as the key step into the new era in plant biology, in which we can design plants that use natural resources efficiently, are resistant to various pests, and are more productive. These products of plant biotechnology (which is itself a product of the basic plant sciences) promise to be the tools that would help us effectively manage our critical resources (land and water), while still fulfilling the needs of the growing human population.

This lab course is designed to introduce the student to the variety and complexity of plant life, and to plant interactions with other living organisms. We will take an observational as well as an experimental approach to understand plants. First, we will study seed plants particularly as they relate to mankind. Second, we will study briefly the biology of non-seed plants, such as ferns. Third, we will explore organisms that were traditionally considered plants, but are now classified into other kingdoms (some protists and fungi)[2]. In the course of the semester, we will also conduct experiments to study plant growth and development, and plant interactions with other organisms. These experiments are designed to teach students to think critically, to design and execute experiments to answer specific questions, and to communicate the results effectively.

Doing good science

Good scientists have a common set of qualities that are acquired from experience and practice.

Good observational skills. Good science stems from making keen observations. In many lab exercises, you will be expected to observe several slides and specimens and make drawings. Carefully observe each slide or specimen. Note the orientation and proportions of various structures, especially on slides. Make neat (not necessarily artistic) and scientifically correct drawings in pencil. Drawings must be of precisely what is seen, not what is supposedly seen. Instructors will

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[1] Raven *et al.* 7e is required for the laboratory and it should be consulted to flesh out these intentionally brief notes. (It is also required for BOT 3015, regardless of the instructor or semester. Students in BOT 3015 who wish to take BOT 3015L later are asked to retain the textbook.)

[2] Students currently taking BOT 3015 or who have taken Outlaw's version of BOT 3015 will have his class notes. Others may download relevant passages from http://www.southernmatters.com/ For sake of economy, there is minimum redundancy between these notes and the lecture notes. Here, for example, you should refer to the class notes for modern classification schemes. Note that Powerpoint presentations are available; the user name is <guest> and the password is <plantsarecool>.

check during the exercise and compare drawings with what is under the microscope. Analyze parts in relation to their names; this analysis will help you remember them better. Keep your eyes open and your curiosity alive. Note anything unusual that you may observe, even if you are not asked to label it.

Good problem-solving skills. Good science is all about solving problems. This skill is especially important when you design experiments. First, identify the question(s) you seek to answer. Second, design an experiment or experiments that will test your ideas. Include required controls in your experiment. Finally, if the results are inconclusive, devise a new plan.

Good organizational skills. There is no substitute for being neat and organized in experimentation and analysis. Have your tools and materials ready before you start an experiment, so that you do not flounder during an important step. Outline procedures before you start, and check each off as you follow them (an example will be given later).

While analyzing data, list additional questions that you could answer from the experiments that you conducted. Organize your data, and select the most appropriate representation of the data (A table? A line graph? A bar graph? What should the axes on your graphs represent? &c.).

Good record-keeping skills (maintaining a lab notebook). Though underappreciated by novices, good record-keeping is imperative to doing good science. In BOT 3015L, the importance of the notebook is emphasized. Thus, each student will maintain a notebook, and record his/her observations (notes/drawings) during each lab period. In addition, all questions should be answered in the notebook in complete sentences. The first pages of each notebook are reserved for a table of contents.

Broadly, two types of labs occur in this course:

1. Observational labs. Entries in your notebook before the laboratory are not required. After examining specimens/slides, observations made during lab will be recorded (according to the manual and drawing list). Drawings should be in pencil on unlined, right-hand pages of your notebook and about ½ the size of the paper. Include a clear description of what is drawn, including such things as scientific and/or common name of specimen, what type of section it is (e.g. cross section, longitudinal section, whole mount, etc.), magnification (total (eyepiece x objective)), what type of tissue (e.g. leaf, root tip, etc.), of objective of looking at the specimen (e.g. visualize mitosis, vascular system, comparing dicot and monocot root, etc.). Always include any procedure that was followed to obtain or visualize the sample. All drawings should be drawn as seen in the microscope. Use close and accurate observation. Label drawings with arrow-free pointers and write in normal orientation (horizontal plane and right side up). Notice shape, size, contents, patterns, and movement. Parts of the specimen should be drawn to scale and a scale bar should be included when possible. An example of a good drawing can be found at http://www.zoology.ubc.ca/courses/bio332/Images/amoeba1.jpg. Note that although the details are not drawn throughout the whole specimen, there is detail as seen in the microscope and notes describe where and what detail is not drawn. All of the parts are drawn, although repetitive parts do not need to be drawn. Now compare to http://www.zoology.ubc.ca/courses/bio332/Images/amoeba2.jpg.

2. Experimental labs. Experimental procedures should be entered into your lab notebook before the lab.

    a. The first pages of each notebook are reserved for a table of contents.

b. The right-hand pages are used to record protocol, solution preparation, methods, and results (*e.g.* drawings and observations).

c. The left-hand pages are for planning and interpretation. There, summarize a previous experiment (e.g., with a graph and a short statement), outline the next experiment, make a note of literature citations, record calculations, etc.

d. Protocols should be recorded before the experiment on the right-hand pages. The system to ensure that protocols are followed in this course is to place a check by each task as it is accomplished. A statement such as "experiment like pg 10" is inadequate. A typical protocol can be recorded as shown on the following:

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13 June 2005

Treatment of *Brassica rapa* plants with GA

Time: 10:05 am

_√__ Measure plant heights

1. _20_ mm
2. _40_ mm

_√_ Apply 20 µl 100 µM GA to 1st leaf of plants 1, 2 and 3.

___ Apply 20 µl 10 µM GA to 1st leaf of plants 4, 5 and 6.

____ ……..etc…….

Even now, your experiment is not over. Analyze your data. Discuss your interpretation with the TA. Write out your protocol for the next experiment.

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Good writing and communication skills (writing a scientific report). This essential skill is learned by practice. A scientist must effectively communicate his results and their implications in a clear, precise, and scientific manner. A substantial cost is associated with creating new knowledge that merits publication in a journal. In brief, present your work carefully if you expect it to be read. Only work distilled to essentials can be published. (In other words, a scientific report is not a diary.) Understanding the importance and costs of communicating science puts one well on his way to creating a good report. Of course, the best way to learn to write is to read, and then write for the purpose at hand.

In summary, make your point in the most straightforward manner. There is no room for personal style, rambling, or inaccuracies in scientific writing.

1. Organization of a long lab report (GA report).

a. Title. Thousands and thousands of people will read your title. How many will be stimulated to read your paper? Make every word count. Here's a bad title: "Effect of temperature on plants." Here is a good title: "The temperature threshold for dormancy induction in *Malus* seedlings." Remember that generally articles are not even retrieved by people; computers do it on the basis of key words, words in the title, author, or citations. "Effects of..." or "Studies

on..." are meaningless expressions. On the other hand, "dormancy" will alert the searcher right away. No one will retrieve the first title because there is little chance that the study will be relevant to the researcher.

b. ii. Abstract. If the title is crafted well, readers might be attracted to the abstract, which is a summary of the essential pieces of information and conclusions reached. Brevity is of the essence. For example, methodological details, unless important to the conclusion, should be omitted. References to previous work usually should be omitted. A reader will use the abstract to determine quickly whether the full document has relevancy to his or her interest. The task is to put into 150-200 words the major elements of a scientific paper, viz., (a) the question investigated and why, (b) the general methods employed, (c) the essence of the results, and (d) the principal conclusions.

c. Introduction. The purpose of the introduction is to state the nature and the scope of the problem. Why was it interesting and worthwhile to do? You should provide scientific background; the reader in your target audience should not have to consult other work for a general understanding of your paper. What will the data permit the reader to conclude? Chart the course that will follow; the introduction is a kind of map—scientists are busy, make it easy for them.

d. Materials and methods (written in past tense). Full details that permit a competent colleague to reproduce your work are required. [Plant used, materials used, description of experimental set-up, all methods in detail, (details of what you did and when, what special care you took in what step, etc.)]

   The Materials and methods, and the Results are the cornerstones of a scientific paper. It is fine to reference other work that is readily available or common knowledge. Everyone will know "according to the method of Lowry et al. (1951)," but for most methods, even if you reference, you will want to give the reader a notion of the approach, e.g., "Potassium was determined by flame photometry (Doe and Doe, 2005)." Note the method of inserting a citation within the text.

e. Results. This is the new knowledge, your contribution. It is not a place to rehash methods. It is not a place to express opinions. Predigest your data. The reader is not interested in the results on the day that you broke your measuring cylinder. It is your responsibility to edit your work—no one has the interest or time to go through the hundreds of measurements you may have made. Include a representative set experiments or, if appropriate, statistical representations, such as the mean and associated errors, and other statistical analyses. Remember that the other parts of the report are included as support for the results. Use a written section to describe the results of the experiment and to support and refer to any graphical representations. For figures and tables, include a written legend that describes what is being represented.

f. Discussion. The discussion is perhaps the hardest part to write, as it requires “the grand vision.” What do the data mean? How do your results and all other results provide a more complete interpretation of how the world works? Your task here is to discuss, not simply to restate, the results. Do your data fit with those of Jones et al. (2004)? Are your results internally inconsistent? Point to the strengths and weaknesses of your work. Are there alternative interpretations? When you finish writing the discussion, which you should do last, decide whether to throw the paper away or try to get it published.

g. Literature. Research starts with the literature, and it is fitting that the report should end with it. Restrict the number of references to the minimum. No one cares how many papers you

have read. For generally accepted ideas (*e.g.* mass action), use no reference. For accepted ideas more or less peripheral to the central issue of your work, provide a reference to the secondary literature (reviews). Then, the choice is harder. Do not simply cite a paper because it conveniently supports your work. Do what is right, fair, and accurate.

Cite a paper in the reference list as follows: Doe JJ, Smith SM 2005 Process methods for large-scale production of plant antibodies. J Expt Bot 5: 25-34.

In summary, your lab reports should be set up like a research article in a scientific journal. Although journal styles vary somewhat—the Abstract might be called a Summary or the Methods may be placed after the Discussion—all papers have in principle the elements listed above. The quality of your reports and the explanation of results are as important as your results themselves. In other words, despite your best efforts, you may not obtain interpretable results. In this class, a report is required; in the real world, those results are not of interest, but may form the basis for further investigations. Remember, an investigator is not out to prove a point. She or he develops a testable hypothesis, executes a research plan, and decides whether the results support the hypothesis or invalidate it.

2. Organization of a short lab report (Two reports).

Two short experiments will be conducted in addition to the GA experiment. The short experiments will be conducted in pairs or small groups depending on time and material constraints. At the end of each short experiment, you will obtain data, either in the form of numbers or observations. Although the experiments are performed collaboratively, reports are products of each student.

Each short report should be 1-2 pages long (single-spaced, 12-point font), prepared professionally (word processing and spreadsheet applications), and contain the following sections:

a. Title. Limit to one sentence, preferably reflecting a result. E.g., "Malate accumulation in the guard-cell apoplast modulates stomatal aperture size."

b. Goal of the experiment. Limit to one or two sentences. E.g., "Exogenous malate activates the guard-cell anion channel, which is the cardinal event in stomatal closure. Here, I determined the *in vitro* malate concentrations required to diminish aperture size and compared that with *in planta* malate concentrations in the guard-cell apoplast."

c. Scientific background. Limit to about seven sentences. What is the importance of this experiment? What is already known about the various components of the system in question? What particular aspects of this system are you interested in? Why? E.g., "Carbon dioxide for photosynthesis is acquired by plants from the atmosphere through stomata. However, water vapor is lost through stomata, too. Therefore, the aperture size must be adjusted to permit photosynthesis, but avoid deleterious water loss. Drought induces malate concentration increase in the leaf apoplast and malate induces stomatal closure. Here, we report that under certain conditions, sufficient malate accumulates around guard cells themselves to diminish aperture size, which was previously unknown."

d. Hypothesis. Limit to one or two sentences. The hypothesis is one of the most critical elements of scientific inquiry. Formulation of a hypothesis requires a comprehensive knowledge of the area of inquiry, the ability to distinguish important from unimportant questions, and the conceptual skills to devise a question that can be tested with proposed approaches and which yield an unambiguous answer. The hypothesis and goal(s) obviously overlap. Demonstrate your rationale for the hypothesis.

e. Materials and methods. Limit to about seven sentences. Briefly describe in past tense how the experiment was conducted. Although it is tempting to think that this section is *pro forma*, in fact, this section requires a great deal of judgment concerning the appropriate amount of detail. Consider, for example, the present case. Do you simply report the concentration of malate used, or is it appropriate to report the pH of the solution, the counter ion of malate, the source (if malate is often contaminated with a stomatal effector), the temperature (if the effect of malate is temperature sensitive), the time of day (if there are diurnal patterns in stomatal sensitivity to malate), the precise growth conditions (as it is known that sensitivity to stomatal effectors is related to plant history) . . . and the list could go forever. Obviously, choking a report with trivia is not desirable, but the elements required for interpretation are. In a word, judgment.

f. Results. Present your numerical data, as appropriate. Use proper units, and label the data clearly. Of course, tables and figures must include a detailed, written legend. Consult any scientific journal for a guide. The written section of the results should describe and refer to the tables and figures.

g. Discussion. Limit to about five sentences. Do your data fit your hypothesis? What do the results tell you in terms of the functioning of the system? What is your final conclusion? What subsequent studies are of interest?

Crop investigation

The top food crops in the world are, in approximate order, wheat, rice, maize, potato, and barley and account for about 70% of the total calories consumed by humans worldwide (3). Our lives are sustained by agriculture. Agriculture became the primary means of procuring food about 10,000 years ago (less than 1% of human existence); until then, humans were hunters and gatherers (1). The domestication of crops impacted human population, history, and society. With the introduction of agriculture, humans became sedentary and families grew (maybe because sources of food were more reliable or to help maintain the farm). In addition, sedentary life allowed a few people to produce food for the forming villages, while others could focus on increasing human knowledge and invention resulting in increases in population. The numbers below demonstrate population growth prior to agriculture through today (4).

10, 500 years ago 5 million people worldwide

↓ 8000 years

~130 million people

↓ 2150 years

~500 million people

↓ 300 years

~2500 million (2.5 billion)

↓ 50 years

~6000 million (~6 billion)

Although it is debatable how much of the growth in population is due to agriculture, it is certain that agriculture has and will be responsible for the sustenance of the population. Of all proteins consumed by humans worldwide, plants contribute ~70% and animals ~30% (4). Many of the more than six billion people worldwide are starving and malnourished; however, this seems to be due to problems in food distribution rather than production (1). Unfortunately, economics supercede need in food-distribution decisions within and across countries. Advances in agriculture to sustain our growing population include irrigation techniques; protection from pests; genetic improvements resulting in better yield, quantity, and quality; domestication of new crops; and genetic engineering (4) (transfer of one or a few foreign genes into plant cells (for perspective, one model plant for genetic studies, *Arabidopsis thaliana*, has ~25,700 genes)).

In addition to human population, domestication impacts ecology and landscapes. For example, after crop domestication, animal domestication followed. Large herds of grazing animals destroyed many pastures containing domesticated and native plants in the Near East (Lebanon, Syria, Turkey, Iran, Iraq) resulting in desert formation (4). Of the total land area of Earth, 11% is used for crops, 24% for pastures, and 31% is forest (1).

By cultivating plants, humans have greatly impacted and directed the evolution and characteristics of crop plants by deciding which plants grow where, protecting useful plants from diseases, and helping to evolve new species and characteristics of species that would not survive without human intervention (1). Some may refer to domestication as “directed evolution” by intentional selection instead of natural selection. For example, natural selection on maize would favor seeds (kernels) dispersing, but intentional selection for agriculture favors seeds remaining on the cob (similar for

cereal crops such as wheat). Other characteristics that farmers often select for include loss of dormancy, loss of fruit production for those crops that humans use for roots or other vegetative tissues (e.g. potatoes), loss of seeds for those crops that humans use for fruits (e.g. bananas), and increased size of the organ of interest (e.g. compare the corn cob of wild corn with that you see in the supermarket) (1). As an example of the degree to which human selection can impact plant characteristics, all of the following have been domesticated from the same species, *Brassica oleracea*: cabbage, kale, kohlrabi, Brussels sprouts, cauliflower, and broccoli (3).

In addition to food, plants are cultivated for many purposes including oil, pesticides, perfumes, shelter, fuel, cloth, tools, and drugs. Interestingly, until about 150 years ago, botany was a branch of medicine. Today, ¼ of the prescriptions written in the USA contain at least one product that has been derived from a plant; plants are used even more commonly in other countries. Plants have been and are used to treat every system of the human body (2). Take a look around; there are few objects around you that are not derived from plants.

Bibliography:

1. Chrispeels, MJ and Sadava, DE. *Plants, Genes, and Agriculture.* Boston: Jones & Bartlett, Inc., 1994.
2. Lewis, WH. *Medical Botany.* New Jersey: Wiley & Sons, Inc., 2003.
3. Outlaw WH Jr BOT 3015 Class Workbook
4. Raven, PH, Evert, RF, and Eichhorn, SE. *Biology of Plants 7th edition*. New York: W.H. Freeman and Company, 2005.

Research and presentation

Now, you will be investigating, in groups of two or three, one of the crops listed below.

maize	banana	okra	flax
wheat	yam	barley	black pepper
soybean	rice	cacao	
peanut	sweet potato	coffee	

In your notebook (or on a sheet of paper that will be incorporated into your notebook), answer the following questions using complete sentences. Each student is responsible for answering all questions, but the research and presentation are group efforts. As references, utilize texts provided by the instructor and the Internet. *Plants, genes, and agriculture*, cited above, can be found as an electronic book (ebook) through the FSU library online catalog. Include citations indicating from which reference the information came and a list of references cited. (One way to do this is to number the references and then cite elements of information using the number as in the introduction above.) Finally, each group will give a short oral presentation (~three minutes) about the answers to the questions below to the rest of the class. All group members should present some information and information should be presented in a logical order. Please, also introduce yourself during the presentation.

Questions

1. What is/are common names for your crop?
2. What is the genus for your crop?
3. What parts of the plant are used by humans and for what purpose(s)?
4. From what region of the world did the crop come?
5. For about how long has your crop been used by humans?
6. What countries grow and/or export the crop today?
7. What are some interesting facts about this crop that you found?

Chapter 2

Introduction to Eukaryotic Autotrophs and Osmotrophs

Objectives

Use of the microscope. Identify the parts of the compound transmitted-light microscope and understand their functions. Know the available magnifications. Define resolution. Understand the difference between magnification and resolution. Compare sizes of various biological structures that are mentioned in this chapter.

Plant cells. Prepare a wet mount. Observe plant cells. Identify plant-cell components that are common to eukaryotic cells and those[3] that are unique to photoautotrophs. Contrast plant-cell and animal-cell structure.

Cytoplasmic streaming. Explain the role of and molecular basis for cytoplasmic streaming.

Plasmolysis and Osmosis. Define plasmolysis and explain osmosis.

Plastids. Outline the function of chloroplast and amyloplasts, relying on notes from prerequisites as appropriate. Explain the concept of staining and outline the procedure used to visualize starch.

Cell Cycle. Explain the general functions and outcomes of mitosis and meiosis. Describe the stages of mitosis sufficiently to identify them on the basis of chromosome appearance. Distinguish mitotic metaphase and meiotic metaphase I.

Use and Care of the Light Microscope

The most obvious function of the microscope is to magnify small objects that are otherwise invisible. Various innovations have been made in light microscopy, but generally resolving power is the most useful property. Resolving power permits the user to distinguish two parallel lines. Limited by physics, the best ordinary modern compound microscopes allow distinction of objects that are as close as 0.2 μm (0.2×10^{-6} m). In other words, if two lines are < 0.2 μm apart, they will appear as one line. Instructional microscopes are not this good, of course, but have a resolution of ~1 μm. Remember, the resolving power—not raw magnification—is the important parameter; magnification without resolution is empty. Given BOT 3015 prerequisites, this section will provide only a brief review and pertain only to ordinary transmitted-light microscopes.

Microscope components. A base supports the microscope and houses the light source (see figure). Light from the source is concentrated on the stage by a condenser lens, and passes through the specimen placed on the stage. Next, the light passes through the objective lens, which is housed on the nosepiece, and provides most of the magnification. Light then passes through the body tube, into the eyepiece lens (or ocular), and eventually forms an image on one's retina.

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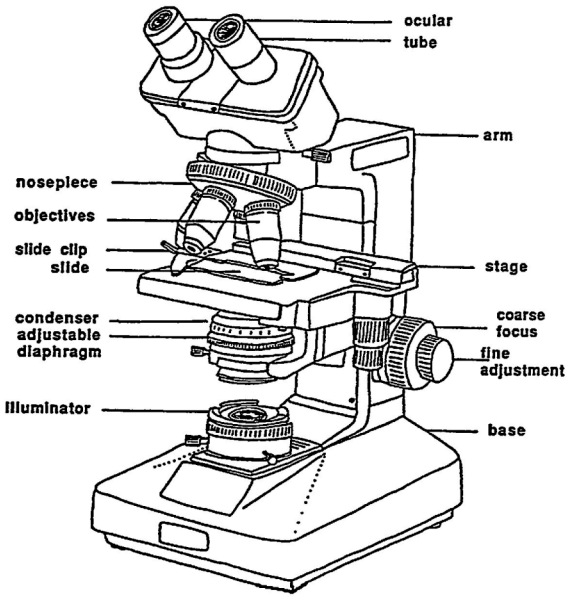
[3] The singular does not exclude the plural and the plural does not exclude the singular. In other words, obtuse grammatical constructions will not be made in order to be strictly correct with regard to number.



Use of the instructional microscope. This refresher section applies specifically to the BOT 3015L microscopes and also provides a baseline for the care of any scientific instrument.

1. Retrieving the microscope. Remove the dust cover (which should always be on the microscope in storage). Grip the microscope with one hand holding the arm and the other, the base.

2. Routine cleaning. Develop a habit of cleaning the lens each time the microscope is used. Only use lens paper. (Other products are not lint-free and may scratch the lens.)

3. Powering the microscope. Plug in the microscope and switch on the light source.

4. Initial Settings. Rotate the nosepiece until the detent for the low-magnification lens (4x) is seated. (The other objectives provide 10x and 40x magnification, respectively.) Place the slide on the stage and secure it using stage clamps. The lens must be a certain distance ("working distance") from the object. At the working distance, a property of the optical system of the particular microscope, the object is in focus. Observing from the side of the microscope, slowly turn the coarse adjustment until the objective is near the slide. Use care to avoid ramming the objective onto the slide. Then, looking through the eyepiece, turn the coarse focus knob to move the objective away from the stage until the object comes into focus. Use the fine focus adjustment knob to sharpen the focus.

5. Light adjustments. Adjust light intensity by altering the aperture of the diaphragm, which is mounted in the condenser housing. The condenser is set properly after a specimen on the stage is in focus. Then, place a thin object, such as a dissecting needle, on top of the illuminator and raise or lower the condenser until the object is in sharp silhouette.

6. Increasing magnification. Focus with the low-power objective and then—watching from the side to avoid damage to the microscope—rotate the nosepiece until the higher-power objective clicks into position. The specimen should be approximately in focus ("parfocal"). Bring the specimen into sharp focus by adjusting the fine-focus knob. Total magnification is approximately equal to the product of the magnification due to the objective and the magnification of the eyepiece.

Cells of a Plant, An Advanced Eukaryotic Autotroph

Eukaryotic cells contain a double-membrane-bound nucleus and membrane-bound organelles. These structures are suspended in cytoplasm, which is delimited by a plasma membrane.

Plant cells contain the basic components of a typical eukaryotic cell. In addition, they contain several specific structures[4]. Several types of plant cells will be examined in this exercise. Each type exemplifies a characteristic of plants such as the cell wall, plastids (chloroplasts and amyloplasts), and a central vacuole.

[4] Fig. 3-3, Fig. 3-7, Fig. 4-1

For ordinary transmitted-light microscopy, material must be mounted in a medium and covered with a flat cover slip to prevent optical distortions and preserve resolution. The medium for permanent slides in BOT 3015 is resin, and for temporary, student-prepared slides, water ("wet mount").

Preparation of wet mount—specimen originally suspended in aqueous solution. A water drop containing the specimen (such as microscopic algae) is placed onto a slide that is maintained in a horizontal position. Use of a coverslip inhibits drying and flattens the preparation (an optical necessity, and also allows lateral movement of the slide without contacting the objective). The coverslip should be placed onto the drop of water by lowering one side of the coverslip so that it touches one side of the drop at a 45° angle first. This procedure minimizes the trapping of air bubbles under the coverslip. (Viewed through the microscope, air bubbles are round and have a dark outline.) If the preparation begins to dry before the observation is completed, add water to the edge of the coverslip. Conversely, blotting the edge of the coverslip with tissue will remove water.

Preparation of wet mount—specimen not originally suspended in aqueous solution. A water droplet is first placed on the slide and the specimen (such as an epidermal peel) is placed onto the water. If the specimen does not sink, a small droplet of water is added on top of it. Otherwise, the procedures are as described above.

Specimen 1: *Hydrilla* Leaves (observation of a living plant cell, cell walls[5], chloroplasts[6], and cytoplasmic streaming)[7]

1. Wet mount. Remove a young *Hydrilla* leaf and make a wet mount of the whole leaf as described above. Place the slide on the microscope stage and observe under low magnification.

2. Observation of cell wall. Observe the rows of rectangular cells. Note that each cell is surrounded by a thick cellulosic cell wall. The wall is outside the plasma membrane, which is below the resolution of light microscopes.

The cell wall serves several functions (strength and structure, intercellular transport, communication, and a barrier against invasion by pathogens).

3. Observation of chloroplasts. Observe and draw the cells and chloroplasts at all magnifications. Note that some chloroplasts are moving because the cytoplasm is streaming and carrying the chloroplasts along.

4. Label cells, chloroplasts, and cell wall.

A chloroplast is a type of plastid, an endosymbiotically derived ~2-µm organelle that converts light energy into stable chemical energy, primarily through the reduction of CO_2 to organic form.

Cytoplasmic streaming is common in plants and mixes the cytosol, thus facilitating transport. The mechanism that drives cytoplasmic streaming includes two commonly known proteins, actin and myosin.

[5] pp. 52-58
[6] pp. 41-44
[7] pp. 38-39

Specimen 2: Onion[8] Epidermal Cells (observation of cell walls and vacuole[9]; and inference of the plasma membrane by plasmolysis)[10]

1. Wet Mount. Cut a red onion bulb into eighths and remove one of the fleshy-leaf pieces. Break the leaf by bending backward. With forceps, remove an epidermal strip and make a wet mount.

2. Observations. Observe the red, rectangular cells under the low-power objective. Note, as before, the cell wall and imagine the plasma membrane that is appressed to it. Observe the nucleus, which appears as a dense body in the translucent cytoplasm. Draw as under 10x and label the large red-stained central vacuole and cell wall.

A central vacuole is a hallmark of plant cells and sometimes occupies > 90 % of the total volume of the cell. The vacuole is a metabolically inaccessible compartment: it sequesters toxins; stores sugars, ions, and other substances; digests some substances; and buffers the cytosol against fluctuations of Ca^{2+}, a signal ion. A red onion is used in this exercise for illustration as the vacuoles contain anthocyanins, which also impart color to flowers. The vacuolar membrane is called the tonoplast, but it is too thin for resolution by light microscopy.

3. Experimental plasmolysis. Remove the slide from the microscope and remove the coverslip. Using forceps, transfer the epidermal tissue to the surface of a strong salt solution (1M KCl) that is in a Petri dish. After 2 min, transfer the onion epidermal tissue back to the slide, and observe and draw it under the microscope at 10x. Remember to record, in your notebook, the procedure you performed to obtain the sample.

Water potential[11] is a physical-chemical term that allows one to quantify the propensity for net diffusive water movement. Typically, two forces are involved. First, water moves from a region of lower ratio of solutes:water to a region of higher ratio. Second, water moves from a region of higher hydrostatic pressure to a region of lower hydrostatic pressure. The net effect of these forces determines the direction of water movement. In the present case, the very high ratio of external solutes: water (KCl solution) "drew" water out of the cells. At first, higher hydrostatic pressure inside the cell also contributed to water egress, but once the membrane pulled away from the wall (plasmolysis), the pressures inside the cell and outside were equal, so only solute content was a factor. (Again, use of a pigmented vacuole allows easy observation of the cell's shrinkage.)

Specimen 3: Potato Tuber[12] Cells (observation of cell walls and amyloplasts)[13]

1. Wet Mount. Using a razor blade, make a very thin slice of potato tuber and place the slice on a slide. Add the coverslip as usual and observe. Blot some of the water off of the slice, add a few drops of I_2KI onto the slice, observe at 10x, and draw at 40x.

2. Observations. Observe and label the cell walls, as before. Observe and label amyloplasts, the brownish purple plastids, organelles, that store starch.

Staining a specimen is a common procedure in microscopy. A stain (like I_2KI here) selectively increases the contrast of a selected cellular component or activity (like starch here).

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[8] Fig. 25-43; p. 577
[9] p. 46
[10] pp. 74-77
[11] Fig. 4-5
[12] Fig. 25-42, pp. 576-577
[13] Fig. 3-12, pp. 42-43

Cell Division in Plants[14]

The cell cycle[15]. Growth of multicellular organisms such as plants results from nuclear division (≡mitosis[16].), cell division (≡cytokinesis, which is discussed in BOT 3015) and cell expansion. Cell multiplication follows a prescribed sequence, the cell cycle, which has two phases: interphase and mitosis. Mitosis and cytokinesis (which are generally synchronous in plants) result in the formation of two identical daughter cells. Haploid, diploid, triploid, tetraploid . . . cells can divide mitotically.

Meiosis[17] **and syngamy**. Sexual reproduction involves the fusion of two haploid gametes (≡syngamy) to form a diploid zygote. Thus, the essence of sex is alternating meiosis (≡reduction division—one diploid cell forms four haploid cells; one tetraploid cell forms four diploid cells . . .) and karyogamy (≡nuclear fusion, to restore the diploid condition). The marvelous outcome is segregation of traits and independent assortment[18], Mendel's two principles. Although the meiotic mechanism itself is generally similar among sexual organisms, the timing of meiosis and karyogamy varies dramatically[19]. BOT 3015L does not address the mechanism of meiosis (see BSC 2010/2011) in detail.

Mitosis. During interphase, each chromosome doubles so that each comprises two identical sister chromatids, which are joined at the centromere. Mitosis, which usually requires less than 1 h, begins when the chromosomes condense and thus become visible when stained. The replicated bipartite mitotic chromosomes are divided equally to daughter cells, as implied above. In the following procedures, observe the characteristic stages of mitosis in a root tip. Importantly, note that mitosis is a continuous process, but the observations are static[20]

Specimen 4: Growing root tips of onion (observation of mitosis)

1. Wet Mount. Remove a healthy white root from a green onion. Then, cut off and discard all except 1-2 mm of the apical end, which is retained on the slide. Add 1 drop of 1 M HCl (!!!) and heat gently for 1 min to fix the cells. Blot the root tip with tissue, add a drop of toluidine-blue to stain chromosomes, and gently heat the preparation again for 30 s. Blot away excess stain, and rinse the section with a drop of water. Make a wet mount. Then apply gentle pressure to the coverslip with a pencil eraser to squash and disperse the tip to essentially a monolayer of cells.

2. Observations. Observe the preparation under the 10x objective. (Recall, always set up the microscope with the lowest-magnification objective, then—step-by-step—increase the magnification.) Locate a dividing cell and observe and draw it using the 40x objective. Label chromosomes, cell wall, and stage of mitosis. Repeat until high-quality observations and drawings of cells in at least two of the following stages have been completed[21]:

a. Prophase—condensation of the chromosomes into microscopically discernable bodies, loss of nuclear membrane.

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[14] Summary comparison: p. 161
[15] pp. 58-60
[16] pp. 61-67
[17] pp. 141-143
[18] Fig. 3-39, Fig. 3-40
[19] In BOT 3015 and in subsequent units of BOT 3015L, three basic sexual life cycles will be studied. Now, study thoroughly Fig. 12-15.
[20] An excellent animated graphic of the dynamic process in onion root tip can be seen at http://www.biology.arizona.edu/cell_bio/activities/cell_cycle/cell_cycle.html
[21] Mitotic stages are diagramed on p. 148. Note, as indicated in BOT 3015, *slight* variations (e.g., timing of nuclear membrane disintegration) in mitosis exists among eukaryotes. The descriptions here apply to onion.

b. Metaphase[22]—chromosomes are aligned on the equatorial plane.

c. Anaphase—chromosome division (sister chromatids separate, each becoming a chromosome of the respective nascent daughter nuclei).

d. Telephase—distinct daughter nuclei.

Review Questions

1. What plant-cell component(s) affect plant-cell shape, and how?

2. Which organelle in plant cells is primarily responsible for autotrophy? Give a brief description of the process in plants that makes them autotophs.

3. If a plant and animal cell were both put into pure water, what do you expect to happen to the cells? What differences do you expect between the effects on the cell types and why?

4. Compare one chromosome of a mother cell to a daughter cell of mitosis and compare one chromosome of a mother cell to a daughter cell of meiosis, how are the chromosomes in these comparisons different between mitosis and meiosis?

[22] Note the key difference in metaphase in mitosis in which homologous chromosomes are not paired and metaphase I in meiosis in which pairing of homologous chromosomes facilitates crossing-over. See Fig. 8-7

Chapter 3

Biology of Flowering Plants: Reproduction

Flowers and Pollination

Objectives

Angiosperms. Understand the distinguishing characteristics of angiosperms. Know the differences pertaining to floral structure between and examples of the two major groups of angiosperms, the monocots and dicots.

Flowers. Besides identifying parts of a flower, understand the relationship between structure and function for the parts of a flower. Know the variations and terminology that exist in flower structure (*e.g.* presence or absence of different reproductive parts in the same flower, in different flowers on the same plant, and/or on different plants; symmetry; ovary placement; inflorescences) and how these variations relate to pollination success and evolution.

Pollination. Understand the process of pollination and the importance of the process for completion of the plant life cycle. Be able to give examples of modes of pollination, explain how these correlate with floral structure, and explain some of the advantages and disadvantages for these strategies.

Introduction to Flowering Plants[23]

The angiosperms[24], flowering plants, are the largest and most diverse group of vascular plants (we will study the other category of plants, the non-vascular plants, later in the course). Angiosperms constitute most of the visible vegetation on earth and most of the plants that you are familiar with are angiosperms. Organisms in the phylum Anthophyta (angiosperms), the largest phylum of photosynthetic organisms, range in size from the 300-foot *Eucalyptus* tree of Australia[25] to the aquatic duckweed[26] (family Lamnacieae) of only a few millimeters (imagine how small the flowers are of a plant that is only one millimeter). Primary distinguishing characteristics of angiosperms include the presence of flowers, fruits (seed(s) enclosed in a vessel or carpel, the basic unit of the pistil), and double fertilization[27] (one to produce the endosperm and the other to form the zygote).

Monocots and Dicots. The two largest classes of angiosperms are the Monocotyledones (monocots) with at least 90,000 species and the Eudicotyledones (eudicots or commonly dicots) with at least 200,000 species[28]. Some familiar monocots include grasses (*e.g.* lawn, maize, wheat), lilies, orchids, irises, cattails, and palms. A few familiar dicots, both herbaceous (non-woody) and woody (flowering trees and shrubs), include peanuts and the related legumes, azaleas, blueberries, strawberries, oak, crape myrtle, rose, carrot, and dill. There are several features that can be used to

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[23] pp. 434-435
[24] p. 436. This name comes from two Greek words, *angion* (vessel), and *sperma* (seed). Angiosperms are plants with seeds that are born in vessels (*e.g.* the pod, or fruit, of peas) .
[25] Fig. 19-1
[26] Fig. 19-2
[27] p. 446 and Fig. 19-22 and a subject of the next chapter of the lab
[28] p. 435-436, Fig. 19-3, and Fig. 19-4

differentiate monocots and dicots[29] and include such traits as number of cotyledons (*e.g. mono* (one) *cot* (cotyledon)), function of cotyledons, presence or absence of endosperm at seed maturity, number of flower parts, root structure, presence or absence of secondary growth in the shoot, vascular bundles arrangement, and leaf venation. Monocots and dicots will be contrasted throughout this course.

In your lab notebook, create a table to contrast monocots and dicots. Include at least the characteristics mentioned above. Likely, you will be adding to this table as the semester progresses. Utilize your textbook, the BOT 3015 lecture notes, and any other reliable resources available to you.

A guided, interactive tour of a flower. The presence of a flower is a major distinguishing characteristic of angiosperms. Taxonomists use details of floral structure to identify species; thus, one reason it is important to learn the terminology associated with floral structure. In addition, a deeper understanding of the structure leads to an understanding of the function of a flower. The parts of a flower[30] are, anatomically, modified leaves born on shoots that have evolved as reproductive parts of angiosperms.

Specimen 1: Flower[31] dissection

1. When obtaining a flower, be sure to cut below the receptacle, the part of the flower stalk to which the floral parts are attached.
2. Notice how the floral parts are arranged around the vertical axis of the flower. One floral evolutionary trend is for the parts to move from a spiral arrangement to a whorled arrangement at separate levels.
3. The most recognizable parts of most flowers are the petals; however, the outermost parts of a flower are the sepals. The sepals are collectively called the calyx and are what encase the flower as a bud. Many times they are green and leaf-like, but in some cases look very much like petals, in which case they are called petaloid or are called tepals.
4. Just inside the sepals, you will find the petals, collectively called the corolla. Together the infertile calyx and the corolla are called the perianth.
5. Count and note the number of sepals and petals.
6. Carefully, to keep the other parts of the flowers intact, dissect and keep intact the sepals and a few petals from the flower.
7. In your notebook, create a dissection press with unlined paper. The first page of the flower dissection press will be blank and will serve as a cover. Keep one sepal and tape one sepal to the second page at the top of the page (see diagram step 9). Do the same with petals, taping a couple of them on the bottom half of the page. Label them.
8. Observe the sepal and petal that you saved under the dissecting microscope. Under the taped, lived specimens, describe what you see under the dissecting microscope.
9. You will be setting up a third page of your press, similar to the page with the sepals and petals, for the reproductive parts of the flower, the stamens and carpel. After dissecting away the perianth, the entire structures of the reproductive structures are revealed. First look at the pollen-bearing stamens, collectively called the androecium (*andro* (man) *ecium* (house)), that surround the pistil that is in the middle. The stamens are also called microsporophylls (microspore-producing modified leaves). In most angiosperms, the stamen is made up of a two-lobed anther, containing microsporangia (pollen sacs), atop a filament. Count and note the number of stamens.

[29] Table 19-1 and table in *Angiosperm Anatomy and Selected Aspects of Physiology* of Outlaw's BOT 3015 lecture notes available at http://www.southernmatters.com
[30] pp. 436-437
[31] As you are dissecting and observing this specimen, use Fig. 19-6 as a reference

10. Dissect a few of the stamens from the flower, keep one and tape the others onto the top of a third page of the flower-dissection press in your notebook and label them.
11. Observe the stamen that you kept under the dissecting scope describe (including function) under the taped specimen. While you are looking, dissect into the anther to find the pollen.
12. You are left with the carpel (when fused or single, also termed pistil), collectively known as the gynoceium (*gyno* (woman) *ecium* (house)). The leaf-like megasporophyll encloses one or more ovules. The tip of the carpel (furthest from the receptacle) is the stigma that receives pollen. The pollen grows a pollen tube that travels down the style, which is usually long, of the carpel until it reaches the ovules that are inside the lower part, the ovary, of the carpel. It is within the ovule that the megagametophyte ("female" gametophyte, derived from mitotic cell division of spores (1N and produced from meiosis)), is found. We will look closer at the gametophyte in the next lab.
13. Under the dissecting scope, carefully slice the ovary open to observe the ovules inside. After fertilization, the ovules develop into seeds. When carpels fuse, the ovary is generally, but not always, partitioned into chambers called locules. The portion of the ovary from which the ovules originate and remain attached is the placenta. The arrangement of the placentae, known as placentation[32], varies and is a way for taxonomists to distinguish different species. Leaving a place to tape the opened carpel to the middle of page three of your press (under the stamen section), draw the carpel, details as under the dissecting scope, label (pistil, stigma, style, ovary, and ovules), and describe (including function) the opened carpel.
14. Finally, tape the opened carpel onto the lower half of page three.

Variations in flowers and trends in floral evolution[33]

1. Evolution tends to favor reductions in the number of floral parts[34]. For example, the *Gladiolus* flower that you just dissected has both carpels and stamens, this is a perfect flower; whereas, an imperfect flower is missing one of these reproductive parts. Imperfect flowers may be either staminate, having only stamens and no pistil, or pistillate, having no stamens. Of course, for species with imperfect flowers to reproduce, both staminate and pistillate flowers must exist for that species. For monoecious (*mono* (one) *ecious* (house)) species, such as maize and oak, staminate and pistillate flowers exist on the same plant. For dioecious (*di* (two) and *ecious* (house)), such as *Cannibas sativa* and willow, pistillate and staminate flowers do not exist on the same plant body, but on different plant bodies.
2. As mentioned before, a cyclic arrangement of parts is favored evolutionarily over a spiral arrangement.
3. Evolution tends to favor fused floral parts over free parts. As discussed during the flower dissection, carpels are often fused. One can also find examples of fused petals, stamens, and even fusion of stamens and pistils.
4. Floral evolution has tended to favor bilateral symmetry (irregularity) over radial symmetry (regularity). Flowers have evolved ornately shaped petals to attract pollinators or as guides and landing pads for pollinators. Some intermediates in regularity exist such as radially symmetric shape, but bilaterally symmetric color.
5. There are also evolutionary trends in the insertion of floral parts along the floral axis. In some cases, the sepals, petals, and stamens arise from below the ovary, which is termed superior. When the ovary is below the insertion points of the sepals, petals, and stamens, it is termed inferior. The evolutionary trend is toward an inferior ovary. Of course, there are intermediates between these two conditions.

[32] p. 438 and Fig. 19-9
[33] p. 458
[34] p. 438

In your lab notebook answer the following questions (all answers in your lab notebook should be incomplete sentences) about the flower that you dissected.

1. Is it a monocot or dicot and what determining characteristics did you use?
2. Is the ovary superior or inferior?

Specimens 2 and 3: More flowers

1. Obtain two of each of two more flowers.
2. Count the number of sepals, petals, stamens, and pistils.
3. Dissect away a couple of petals and/or sepals to reveal the ovary.
4. Draw and label the flower with the intact ovary.
5. Make a longitudinal section of one of each kind of flower, including the ovary, draw and label (sepals, petals, stamens, stigma(s), style, ovary, ovules).
6. Make a cross-section of the ovary of one of each kind of flower, draw and label (ovary, ovules).
7. To your dissection press (new page), add sepals, petals, stamens, and ovaries from these flowers.
8. List the traits of each flower that are advanced.
9. List the traits of each flower that are primitive.

Inflorescences[35]

Many plants produce their flowers in clusters called inflorescences. Although there are many inflorescence arrangements, one of the most advanced kinds of inflorescences is produced by members of the composite family such as sunflowers, daisies, and dandelions. What appear to be petals on the edge of a sunflower or daisy are actually individual flowers called ray flowers. Ray flowers are bilaterally symmetrical, pistillate flowers with one enlarged petal that, together with the other ray flowers, attract pollinators to the inflorescence. The central part of the inflorescence usually consists of numerous, small disk flowers, each of which is radially symmetrical perfect flower with tiny petals.

Specimen 4: Inflorescence

1. Observe the inflorescence with your naked eye and identify the ray and disc flowers.
2. Carefully tease out a ray flower and a disc flower. Observe them under the dissecting microscope. Try to find the reproductive structures in each of the flowers.
3. Tape a disc and a ray flower into your press and, beside each, describe the reproductive structures found in each.

Pollination and the co-evolution of angiosperms and insects

Pollination is the process by which pollen grains[36], which contains the microgametophyte, are carried over to the megagametophyte in the carpel or pistil. This process is imperative for completion of the plant life cycle. Plants are sessile; thus, they must depend on external factors to carry out pollination. Land plants accomplish this in two different ways, by wind and by biotic vectors.

Wind facilitated pollination[37] is the major mechanism of pollen transfer in non-flowering seed plants, such as pine, but is also present in angiosperms, such as oak and grasses. Wind pollination can be an efficient means of uniting micro- and megagametophytes when there is a large population of one species in a small area, such as a pine forest or a field of grass. Instead of investing energy into

[35] Figs. 19-7 and 19-8, pp. 458-459 and Fig. 20-9
[36] pp. 442-444 and Figs. 19-14, 19-15, and 19-16.
[37] p. 463

producing showy flowers to attract biotic vectors, wind-pollinated plants invest energy into making large amounts of pollen.

Some flowering plants have evolved flowers that attract biotic vectors such as insects, birds, and some mammals[38]. Flowers can attract biotic vectors by site, smell, and/or rewards such as nectar or pollen (some flowers even mimic, with petal structure and color, a mate of the biotic vector); thus, flowers and their biotic vectors have co-evolved in a mutually beneficial manner. In many cases, this relationship is very specific; meaning that a particular species of plant is only fertilized by a particular animal species. Flowers have evolved specific structures, colors, smells, and rewards that make them most attractive for their vectors, resulting in a fascinating diversity and complexity of flowers. In addition, flowers have evolved specific placement of reproductive parts to maximize successful pollination. Animal vectors evolve structures that enable them to successfully obtain the reward such as the long proboscis of some moths and the long, slender beak of the hummingbird. Thus, the variations in flower morphology that you observe are not random, but are the results of millions of years of evolution during which increasingly complex and specialized reproductive strategies developed.

The earliest angiosperms were insect pollinated, probably by beetles, but they retained the primitive tendency toward having many reproductive structures. Magnolia trees, which are considered one of the most primitive angiosperms, are pollinated by beetles and have superior ovaries in radially symmetrical flowers with many floral parts. Beetles are rewarded during pollination with food, they eat the flower parts, but do not destroy all the fertilized ovules. Beetle pollination is more efficient than wind pollination for this species, but clearly, leaves much to be desired for the plant.

Bees are the main biotic vectors for pollination, but flies, wasps, butterflies, moths, birds, bats, and more are pollination vectors. Often, the association of a flower and its pollinator is quite specific. Perhaps, the most extreme example is that of the wasp-pollinated orchid *Ophrys*. Orchids are among the most advanced angiosperms; the ovary is inferior and stamens, petals, and pistils are fused. In *Ophrys*, the petals closely resemble the female of a certain species of wasp. This resemblance is so strong that male wasps attempt to copulate with the flowers and in so doing, carry pollen from flower to flower. Some plants spend large amounts of energy to attract insects. The arum lily, generates heat that causes the evaporation of volatile chemicals that attract insects to the flower.

Video: Pollination Biology

In addition to the video, you will certainly see pollination taking place around you.

Review Questions

In addition to the questions and writings found in text.

1. What are the functions of a flower and how are these functions important for the survival of a species?

2. For each of perfect and imperfect flowers, give at least one advantage that each has over the other.

3. Based on the function of flowers, why do you think flower structure is diverse across plant families and sometimes species, but evolutionarily conserved within species?

[38] pp. 460-462

4. Why do you think there is an evolutionary trend toward (*i.e.* what are the advantages to) reduced numbers of floral parts?

5. Describe at least one advantage of inflorescences for reproductive success.

6. Some angiosperms invest energy in ornate flowers (e.g. orchids and sunflowers) and others (e.g. grasses and oaks) do not. Give one possible explanation for how they are both successful despite this difference.

Chapter 4

Biology of Flowering Plants: Reproduction

Gametophytes, Fruits, Seeds, and Embryos

Objectives

Angiosperms. Understand alternation of generations. Understand the life cycle of angiosperms. Identify differences in the angiosperm life cycle and that of other plants. Understand micro- and megagametophyte development. Know which parts of the flower give rise to which parts of the fruit. (For example, the ovule gives rise to the seed.) Understand double fertilization and development of the seed and fruit.

Seeds. Understand the advantage of seeds for vascular plants in a terrestrial environment. Contrast the seeds of gymnosperms and angiosperms. Understand structures in seeds and their functions. Contrast the mature seeds of monocots and dicots.

Fruits. Understand the functions of fruits. Know the types of fruits. Understand modes of seed dispersal. Understand the relationship between the modes of seed dispersal and the structures of fruits.

The Life Cycle of Flowering Plants

The life cycle of plants is descriptively termed alternation of generations[39]. The alternating generations are the haploid, gamete-producing generation, the gametophyte, and the diploid, spore-producing generation, the sporophyte. Angiosperms have small gametophytes comprising only a few cells and large sporophytes. In this lab, we will observe and gain an understanding of aspects of the angiosperm life cycle including the microscopic gametophyte generations; seeds, formed from double fertilization and containing the embryo (new sporophyte generation) and endosperm; and fruits (the vessels that encase angiosperm seeds) and other forms of seed dispersal.

Development of the Microgametophyte[40]

The diploid sporophyte generation of angiosperms produces two types of spores through meiosis, one that develops through mitosis into a megagametophyte, which produces the egg, and one that develops through mitosis into the microgametophyte, which produces the sperm (two sperm cells for double fertilization). Both the mega- and microgametophytes are born on the floral organs of angiosperms. The microgametophyte forms within the anthers of the stamens. Each anther has pollen sacs containing hundreds of microspore mother cells[41].

During microsporogenesis, each microspore mother cell undergoes meiosis to produce haploid microspores. These haploid microspores then develop into microgametophytes through mitosis, a process called microgametogenesis. First, the single haploid nucleus of the microspore undergoes mitosis, then, one of these nuclei undergoes mitosis again resulting altogether in three haploid cells. Two of these cells, the sperm (gametes), are within the third cell, called the vegetative or tube cell. Both sperm undergo separate fusion events (syngamy) in double fertilization as will be described

[39] p. 236
[40] pp. 442-444
[41] Fig. 19-14

later. These three cells are the mature microgametophyte[42], the pollen grain. The pollen grain is released from the sporophyte at or before maturity.

Each pollen grain has a protective wall, which causes allergic reactions in some humans. As indicated, the microgametophyte contains gametes that undergo syngamy within the megagametophyte, which, in angiosperms, is contained within the ovary of the carpel. During pollination, the microgametophyte lands on the stigma, an extension of the ovary; then, the tube cell containing the microgametes (sperm cells) grows down the style of the carpel until it reaches the megagametophyte. More about fertilization will be discussed later. We will observe the process of pollen-tube growth.

Specimen 1: pollen grains (try to observe growth of the pollen tube)

1. Place a drop of pollen-growth medium (sucrose solution) on each of two slides.
2. Obtain an anther from at least two different types of flowers.
3. Dust a few pollen grains onto the medium and place a coverslip on top. Label your slides to know which type of pollen is on the slide.
4. Observe each at 10x.
5. After at least 10 minutes, observe and draw the pollen, which may be growing a pollen tube, again under 10x. Make comparisons with a partner.
6. Answer the following questions in your lab notebook.
    a. Do all pollen grains develop pollen tubes uniformly?
    b. Some pollen grains readily make pollen tubes in a nutrient solution. Do you think this poses a problem for pollination/fertilization specificity? Explain why or why not.
    c. What are possible mechanisms that plants may have evolved for pollination/fertilization specificity?

Development of the Megagametophyte[43]

The diploid sporophyte generation of angiosperms produces two types of spores through meiosis, one that develops by mitosis into a megagametophyte, which produces the egg, and one that develops by mitosis into the microgametophyte, which produces sperm. Both the mega- and microgametophytes form within the floral organs of angiosperms. The megagametophyte is born within the ovules, which are within the ovary, the base of the carpel. There may be one or many ovules per ovary. (Some ovaries have up to several chambers, each one with many ovules, that arise from carpel fusion.)

Each ovule of the sporophyte contains hundreds of cells. During megasporogenesis, one of these diploid cells that is surrounded by many other cells differentiates into a megaspore mother cell (a.k.a. megasporocyte)[44], which, through meiosis, produces four haploid spores. Three of these spores degenerate, leaving one haploid megaspore per ovule.

During megagametogenesis, the haploid megaspore develops into the megagametophyte through three mitotic divisions, which are not immediately followed by karyokinesis, thus producing eight nuclei within the cell wall and membrane of the original megaspore. The eight nuclei become arranged into three groups, one group of three at each end of the megagametophyte, leaving two in the middle. Then, cell walls and membranes develop around each nucleus except the two in the

[42] Fig. 19-16
[43] pp. 444-446
[44] Fig. 19-18a, b

middle. The resultant mature megagametophyte[45] consists of (a) three cells (the egg cell (megagamete) and two synergids) near the micropyle (opening in the integuments that allows the pollen tube access to the megagametophyte), (b) three antipodal cells at the end of the megagametophyte opposite the egg cell, and (c) the original larger cell that contains the two polar nuclei in the middle of the megagametophyte. Thus, the mature megagametophyte generation of angiosperms consists of only seven cells with eight nuclei and is contained within the ovule, surrounded by the integument (made of diploid cells of the sporophyte). The two polar nuclei and the egg cell participate in double fertilization, which is a distinguishing characteristic of angiosperms. Although the mature megagametophyte is a separate generation (a separate plant), in angiosperms, it is dependent on the sporophyte generation for nutrition and protection, essentially, a plant within a plant.

Specimen 2: Prepared slide of cross section of *Lilium* ovary (to observe ovules containing the megagametophyte with egg cells)

1. Before observing the slide, sketch a carpel indicating the stigma, style, ovary, and ovules. Draw a line that demonstrates a cross section of the middle of the ovary
2. Observe, draw, and label the specimen under 4x. This ovary has six chambers or locules (resulting from carpel fusion), each one contains one ovule. Answer the following in your notebook: based on this observation of the ovary, is *Lilium* a monocot or a dicot?
3. Observe one of the ovules under 10x. Do you see a megagametophyte? If not, move to another ovule until you locate one.
4. Draw an ovule containing a megagametophyte at 10x. Label the ovule and megagametophyte. Label, or indicate where you expect to find, the egg cell and the polar nuclei. Indicate the ploidy level of each of the labeled structures.

Double Fertilization: Development of Seed and Fruit

So far in this chapter, we have learned about the development of the micro- and megagametophytes. The gametes of these haploid generations undergo syngamy to form a zygote, the new diploid sporophyte generation[46], which, in all plants, develops into an embryo.

After pollination, the microgametophyte is recognized by the stigma. The stigma provides the necessary environment for growth and penetration of the pollen tube down through the style to reach the ovule-containing ovary[47]. The megagametophyte is enclosed by the integument (of the maternal sporophyte generation). The pollen tube accesses the megagametophyte through the micropyle, an opening in the integument, and releases the two sperm cells into the megagametophyte.

One sperm cell fertilizes the egg cell resulting in a diploid zygote (new sporophyte generation). The other sperm cell fuses with the polar nuclei in the middle of the megagametophyte resulting in a triploid endosperm. These two fertilization events are termed double fertilization[48], which occurs only in angiosperms.

After double fertilization, the embryo and endosperm develop within the seed coat, which is derived from the "maternal" sporophyte. The ovary and, sometimes, associated tissue develop into the fruit. During seed development, the triploid endosperm grows mitotically and accumulates storage nutrients. In dicots[49], the diploid embryo (including the cotyledons) then continues to grow through

[45] Fig. 19-19
[46] p. 236 (Fig. 12-15c)
[47] Fig. 19-20
[48] Fig. 19-21
[49] Fig. 22-3

mitotsis; the growth is supported by nutrients from the endosperm and continued release of nutrients from the seed coat. In contrast, the monocot embryo is small [50], and the single cotyledon only absorbs endosperm nutrients when the endosperm degrades during germination. Thus, comparing monocot and dicot seeds, mature dicot seeds contain larger cotyledons and little endosperm; whereas, monocot seeds have a small cotyledon and much endosperm[51]. The integument, derived from the sporophyte tissue, becomes the seed coat. The ovary, which contained the ovule, develops into the fruit. There is a large diversity in fruit structure, as will be discussed in the next section. Fruits provide protection, nutrients, and, most importantly, a means of dispersal for the seeds that they contain.

See pages 448-449 for a complete review of the angiosperm life cycle demonstrated with soybean.

Specimen 3: Prepared slide of longitudinal section of the *Capsella* ovary with seeds (to observe structures of a mature dicot embryo)

1. Observe under 4x, draw, and label. The whole structure on the slide is the fruit and each small, oval structure within the fruit is a seed.

2. Observe under 10x, draw, and label one embryo-containing seed. Each embryo has a root apical meristem and a shoot apical meristem. Meristems are regions of perpetual growth. Each embryo also has one (monocots) or two (dicots) cotyledons. Label the seed, seed coat, embryo, cotyledons, root apical meristem, shoot apical meristem, and remains of endosperm.

Seed Dispersal

Once the embryo is fully developed, the seed dries out and becomes dormant. The power of seeds as reproductive structures lies partly in their stored food reserves, partly in their ability to remain dormant while awaiting the proper growing conditions, and partly in their adaptations for dispersal. The fruit plays a critical role in seed dispersal. The reproductive strategy of each species is reflected in the structure of its seeds and fruits. Seed dispersal is particularly important because it facilitates invasion of new environments. Obviously, the seed was a remarkable evolutionary adaptation to terrestrial environments.

A fruit[52], which develops primarily from the ovary following fertilization and seed development, may be either fleshy or dry. Fleshy fruits such as apples, oranges, and berries are adapted for dispersal by animals. The animals eat the fruit, but the seeds pass through the animal's digestive tract unharmed and, when they emerge, have not only been transported to a new habitat but have received an application of fertilizer as well! Some dry fruits, such as nuts, are also adapted for dispersal by animals. In nuts, the ovary wall dries out and forms a hard covering around the seed. Nuts are collected by small mammals, which take them to their burrows or bury them, as squirrels do. In the spring, the nuts that have not been eaten sprout. Other dry fruits are adapted for dispersal by wind, such as the winged fruits of maples and dandelions.

Still others have burrs, cling to animal fur and are thus transported to new habitats. Another interesting adaptation is found in fruits that dry out and discharge their seeds explosively. But perhaps the most unusual dispersal mechanism is that of the coconut, which has colonized all of the South Seas atolls by floating from island to island.

[50] Fig. 22-8, 22-13
[51] Fig. 22-7
[52] pp. 466-470

Fruits are generally classified as simple, multiple, or aggregate, depending on the arrangement of the carpels from which the fruit develops.

Simple fruits develop from one carpel or from fused carpels. Simple fruits may be fleshy, dry, or papery. Dry simple fruits may be dehiscent, the ovary wall opens and frees the seeds, or indehiscent, the seeds remain in the fruit after the fruit has been shed from the parent plant. Examples of dehiscent fruits include legumes[53] (*e.g.* peas) and poppies[54]. The most common indehiscent fruit is the **achene**, which is single-seeded. Examples of achenes include the fruits of ashes[55], elms, grasses[56], and strawberries. The fleshy part of the strawberry is a swollen receptacle and the "seeds" are the achene fruits.

Some common simple, fleshy fruits are described below.

Berry. Tomatoes, dates, and grapes are examples of berries. Each carpel typically contains many seeds. The inner layer of a berry is fleshy.

Drupe. The inner layer of the fruit, derived from the ovary wall, is hard and stony and usually sticks tightly to the seed. Peaches, cherries, olives, plums, and coconuts (which are not nuts) are examples of drupes**.** The outer layer of the drupe is often fleshy; in the coconut, though, it is fibrous. The coconut is a monocot and the seed retains its endosperm. In coconut, the endosperm is in two forms: the liquid endosperm (the milk) and the solid endosperm (the meat). The small embryo is hidden under one of the "eyes" of the coconut.

Pome. Apples and pears are more complicated fruits called pomes. The outer fleshy layer is derived from fused sepals and petals surrounding the ovary, while the core develops from the ovary. Strictly speaking, much of what we eat is not the fruit, because it is not derived from the ovary!

Multiple fruits. Multiple fruits develop from an inflorescence rather than from a single flower. Pineapples and mulberries are examples. Pineapples are unusual in that their fruit develops even in the absence of pollination and fertilization. Consequently, these fruits lack seeds. Blackberries and strawberries are not multiple fruits because they are derived from single flowers that contain many pistils.

Specimens 4 and 5: Fruits

1. Observe, draw, and label (ovary, seeds, and endosperm (when visible)) at least two types of fruits that have been cut in cross section or longitudinal section.

Take-home assignment

Obtain at least three seeds of at least one monocot and one dicot to germinate and begin to grow at home. They may be germinated in a cup of soil or in a plastic, sealable bag containing a damp paper towel (be sure it is not too wet or this will be an exercise in decay and fungi). In your lab notebook, record observations every other day. Bring your seedlings to class on the day (two weeks from now) we are scheduled to observe seedlings in class. You are encouraged, but not required, to conduct an independent experiment to understand factors that affect germination.

[53] Fig. 20-22
[54] Fig. 20-21
[55] Fig. 20-23
[56] Fig. 20-24

Review questions

1. How does the genesis of the gametophyte differ from the genesis of the sporophyte?

2. What are two distinguishing characteristics of angiosperms that pertain to seed and fruit development?

3. What are two types of seed dispersal mechanisms and what fruit structures or features facilitate these mechanisms?

Chapter 5

Biology of Flowering Plants

Regulation of Plant Growth by Plant Hormones

Objectives

Plant Growth Regulators. Know the names of the plant growth regulators (plant hormones) and the effects of each on plant growth and development. Know, in brief, the history of the discovery of gibberellic acid (GA).

Model Plant Species. Know the characteristics that make a species, like *Brassica rapa*, a good model organism. Understand how mutants of a well-characterized model species are useful in experimentation.

Experimental Design. Understand important elements of experiments and how they are useful for interpretation of experimental results.

Introduction to Plant Hormones[57] (a.k.a. plant growth regulators)

Hormones are important in the growth and development of higher organisms. Animal physiologists established the term hormone in the early 20th century. A hormone (from Greek *horman* meaning "to stimulate") is a substance or chemical produced in one part of an organism (source) and transported to another part of the organism (target) where it causes specific physiological effects. Plant hormones vary from this definition in that (1) some plant hormones are inhibitory rather than stimulatory and (2) plant hormones can have an effect on the cells that produce them, in addition to other cells, tissues, or organs after transport. Thus plant hormones are often referred to as plant growth regulators rather than hormones.

In plants, as in animals, hormones regulate various physiological processes. In plants, hormones regulate processes such as, but not limited to, seed germination, plant growth and cell division, responses to stresses, fruit development, and controlled tissue death (senescence) (*e.g.* when petals fall off after fertilization).

The classical plant hormones are classified into five main groups[58] based on chemical structure. Notice that there are overlaps in the functions of the following hormone groups, thus exemplifying the complexity, which has been simplified in this introduction, of hormone effects.

Auxins[59]. Charles Darwin and his son Francis conducted experiments[60] that led to the discovery of auxin, the first plant hormones discovered in plants. Auxins (from Greek *auxein* meaning "to increase") are primarily produced in shoot tips and developing seeds and are involved in polarity of the plant root-shoot axis that is established during embryogenesis, cell elongation, cell differentiation, lateral root formation, and fruit formation. Ordinarily, if a flower is not pollinated and fertilized, the fruit will not develop; however, if auxin is applied to the carpels of certain species, fruit can be produced in the absence of fertilization. Fruit development without fertilization is

[57] Chapter 27
[58] Table 27-1
[59] pp. 605-608
[60] Fig. 27-1

termed parthenocarpy. In large amounts, auxins can be toxic and can inhibit growth. This property of auxins is useful in agriculture; some synthetic auxins are used as weed killers.

Cytokinins[61]**.** The discovery of cytokinins began in 1941 when Johannes van Overbeek found that coconut milk (liquid endosperm) contains a potent growth factor that greatly accelerates the development of plant embryos, cells, and tissues in test tubes. Subsequently, the growth factor responsible for this growth was isolated from a DNA preparation (see structure Fig. 27-8) and termed kinetin. Kinetin is one of the plant growth regulators that are collectively termed cytokinins. The term cytokinin was established because cytokinins promote cytokinesis, cell division (Recall that mitosis is nuclear division, so technically speaking, formation of two daughter cells requires mitosis and cytokinesis.). Cytokinins also inhibit senescence. Cytokinins are required for plant cell tissue culture, which is important for horticulture, agriculture, and plant biotechnology.

Ethylene[62]**.** The effects of ethylene on plant growth were first discovered in the 1800s when defoliation of trees along streets was observed around leaks in gas-burning street lamps. Ethylene, a gaseous hyrocarbon, was identified to be the compound in the lamps that caused the defoliation. Ethylene, in most plant species, has an inhibitory effect on cell expansion and growth, promotes senescence, and promotes abscission (shedding of organs such as leaves or floral parts). Ethylene promotes fruit ripening and is commercially useful for this purpose. In addition, ethylene is commercially used to cause leaf abscision (*e.g.* of cotton before picking), to promote senescence (*e.g.* of tobacco to speed "curing" before drying), and to promote flowering.

Abscisic Acid (ABA)[63]**.** Frederick Addicott and associates disovered abscisic acid while studying compounds responsible for abscission of fruits of cotton. At about the same time however, two other groups of scientists discovered the same compound. One group, led by Philip Wareing, was studying bud dormancy in woody plants; the other group, led by van Steveninck, was studying abscission of flowers and fruits from lupine. Despite the name abscisic acid, abscission is more often associated with the presence of ethylene; whereas, ABA is primarily involved in response to stress. ABA is produced during early seed development in most plant species and is responsible for preventing premature germination and ABA levels in seeds decline under conditions that induce germination. ABA is also important in the responses by plants to root stress. When roots experience stresses (such as drought, freezing, or high salt) they produce ABA, which is transported through the xylem to the leaves. Evaporation of water through stomata from leaves helps cool leaves and, most importantly, creates the force necessary to pull water and dissolved nutrients and hormones up from the roots through the xylem to the leaves where photosynthesis takes place. Stomata are regulated by a pair of highly specialized cells, guard cells, that surround the pore. Guard cells respond to ABA, produced by roots under stress conditions, by causing the stomata to close, thus preventing excess water loss. The response by guard cells to ABA is a primary area of research in Dr. Outlaw's laboratory at FSU.

Gibberellins (GA, gibberellic acid)[64]**.** The discovery of gibberellins began when Japanese scientists were studying a diseased called "foolish seedling disease" in rice. Plants with this disease grow rapidly, are pale and spindly, and tend to fall over. The disease is caused by a substance produced by the fungus *Gibberella fujikuroi* which is parasitic on the seedlings. The substance that causes the symptoms was isolated from bean seeds and termed gibberellin.

Gibberellins are important in breaking seed dormancy (contrast to ABA), inducing flowering, stimulating pollen tube formation, and stimulating fruit development. Like auxins, gibberellins can

[61] pp. 608-611
[62] pp. 611-612
[63] pp. 612-613
[64] pp. 613-615

induce parthenocarpic fruits. Application of gibberellin during fruit formation is useful commercially for the production of fruits, such as grapes. Gibberellins also stimulate cell division and elongation, thus explaining the symptoms of the "foolish seedling disease." Gibberellins have been identified in many species (and are believed to be present in all plant species) of plants at various amounts in all parts of plants. There are over 125 gibberellins that vary in structure and activity; however GA_3 is the most thoroughly studied.

Understanding the Plant Growth Regulator, GA, through Experimentation[65]

The question. You are now going to design an experiment and form a hypothesis to answer the question "What are the effects of GA on the growth of *Brassica rapa*?"

Brassica rapa is an agronomically important species that is in the same family as cabbage, broccoli, turnip, mustard, cauliflower, and other familiar vegetables. About one-third of the vegetable oil worldwide is produced by *Brassica rapa*. Generically, the product is rapeseed oil, but special strains produce a higher quality oil, canola (which is a contraction of *Can*adian *o*il *l*ow *a*cid).

Experiment Design. As a group, design an experiment to answer the question (see Materials below). Write and, if you find it helpful to demonstrate, draw your design in your notebooks. Details (how much? how many? how often? where? how? when?) are important. As a general guideline, GA treatments should be minimally once every other day for 10 days. Include in your design what you plan to measure and observe, *i.e.* what data you will collect. Remember to include elements of good experimentation, such as controls and repeats for statistical analysis. Due to time constraints, these experiments will continue for three weeks and, in this time, plants may not flower or produce fruit.

Materials. The following materials are available for your experiment:

- *Brassica rapa* seeds

Paul Williams and his colleagues at the University of Wisconsin have worked for years on the genetics of the Brassicaceae family of plants. Because they are unable to obtain crosses between some species, directly transferring a trait from one vegetable in this family to another is not always possible. However, they are able to transfer the trait to an intermediate plant, one that can be crossed with both donor and recipient. Obviously, time is crucial in science and agriculture, so Williams developed a special strain of *B. rapa* (the intermediate) that can be grown from seed to seed in a very short period of time and consumes few resources, making it a good model species. Like most excellent research scientists, Paul has been enthusiastic about passing his knowledge to students. To make a long story short, the National Science Foundation and many dedicated people have developed hands-on laboratory exercises that have extended the utility of this plant from the research bench to elementary school. FSU adopted this so-called Fast Plant in 1992.

- Rosette (mutant) *Brassica rapa* seeds

These mutants produce less GA, which results in a dwarfed phenotype.

- Culture materials for planting and growing *B. rapa* seeds

- Solutions containing either 0, 3, or 39 μM GA.

[65] Refer to Chapter 1 of the lab manual for tips on "Doing Good Science"

If your experiment design requires other materials, check with your instructor to determine availability.

Hypothesis. Examine your experimental design and predict what you think will happen. Again, write and, if helpful, draw your predictions in your lab notebook.

Procedure and Data Collection. Write in your notebook a step-by-step procedure. Include the detail required for a peer to replicate your work independently. After, the procedure is written in your notebook, execute the procedure as a group. If, as you are executing the procedure, there are any additions or modifications, amend your notebook. Plan to keep a table of data and observations maintained by the entire group with the plants and to keep a record of all data that you as a member of the group collect in your lab notebook.

Because this is a group activity, be sure to organize well and commit yourself to your responsibilities. Failure to carefully, ethically, and responsibly conduct the experiment will result in complete loss of credit for this portion of the lab. All members of the group must have equal responsibility during the experiment. Participation points are part of the grade. Remember to uphold the Academic Honor Policy.

Chapter 6

Biology of Flowering Plants

Anatomy – Seedlings, Meristems, Stems, and Roots

Objectives

Seedling germination and anatomy. Understand meristem structure and function and how meristems are protected during germination. Understand germination. Know the function of the structures of a typical dicot and a typical monocot seedling.

Primary growth and tissues. Know the difference between primary growth and secondary growth. Know the structure and function of the major types of primary tissues and the cells that constitute these tissues.

Seed germination and seedling structure

When a seed is deposited in an environment suitable for germination, several external (*e.g.* temperature, light, and water) and internal factors (*e.g.* gibberellins) break dormancy and cause resumption of embryo growth. The seed coat, which protects the embryo, and seed dormancy, which ensures adequate conditions for growth before breaking from the protective seed coat, are adaptations to a terrestrial environment. During germination, the nutritive tissue is used for the growth of the embryo.

The first structure to emerge from most seeds is the primary root, which enables the developing seedling to become anchored in the soil and to absorb water for metabolism. There are two major types of structural root systems[66], the taproot system (found in all seed plants except monocots) and the fibrous root system (found in monocots). In the taproot system, the primary root persists, gives rise to lateral roots, and is termed the taproot. In the fibrous root system, the primary root does not persist and the main root system develops from roots that arise from the stem. Taproot systems generally penetrate deeper into the soil. The record depth for penetration of roots is ~53 meters (~175 feet) by the desert shrub mesquite (*Prosopis juliflora*).

Roots extend through soil by cell division that takes place at the root apical meristem. Meristems[67] (from Greek *merismos* meaning division) are embryonic cells that retain the potential to divide long after embryogenesis. The root cap, made of parenchyma cells, protects the root apical meristem from abrasion by soil. After the primary root emerges, lateral roots[68] emerge from within the primary root. In addition, most roots form root hairs, extensions of the epidermis, that increase the surface area of the root, thus allowing for increased water and solute absorption.

Emergence of the shoot during germination varies in different plant groups[69]. In some dicot plants, the hypocotyl elongates and bends to form a hook. The shoot apical meristem and cotyledons are at the end of the hypocotyl hook and are therefore protected from abrasion by soil. In addition, the seed coat often remains around the meristem and cotyledons, further protecting them from abrasion. Once the hook emerges from the soil and light is sensed, the hook straightens thus positioning the shoot apical meristem at the top of the shoot and the cotyledons emerge from the seed coat and

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[66] Fig. 24-2
[67] p. 510, Fig. 23-1
[68] pp. 540-541
[69] pp. 506-507, Fig. 22-10, Fig. 22-11

become photosynthetic until true leaves develop and the cotyledons wither or become photosynthetic. This type of germination in which the cotyledons are pulled above soil by the hypocotyl hook is called epigeous. In contrast to a hypocotyl hook, in some dicot plants, an epicotyl hook forms. Because, in this case, the hook grows from above the cotyledons, the cotyledons are not carried above ground with the shoot apical meristem and thus, remain underground. This type of germination is called hypogeous.

In the majority of monocot seeds[70], the endosperm is the stored food. In some monocots, the single cotyledon forms a hook that carries the seed coat and enclosed endosperm to the soil surface, and, in some cases, the cotyledon becomes photosynthetic. In other monocots, such as maize, after the primary root emerges, a sheath-like coleoptile emerges. When the coleoptile reaches the soil surface, the first leaves emerge.

Specimen 1: Dicot seedling[71]

1. Carefully remove a bean seedling from the soil. Be sure to include the entire seedling including the roots.

2. Draw and label the parts of the seedling. Identify the primary root and root hairs (if possible), seed coat, cotyledons, first true leaf, hypocotyl, and epicotyl of the seedling.

3. Answer the following question under your drawing: Does this seed type undergo hypogeous or epigeous germination and, how do you know?

Specimen 2: Monocot seedling[72]

1. Carefully remove an entire young maize seedling from the soil. (Remember that the maize kernel is a fruit, the majority of which is seed, and the external layer that is usually yellow or other colors is derived from the ovary.)

2. Draw and label the parts of the seedling. Identify the primary root and root hairs (if possible), the coleoptile, and the first true leaf.

Primary and Secondary Growth

Meristems. During embryogenesis, the apical-basal axis and radial patterning of the plant are established. Plant development occurs by meristematic activity. Apical meristems are found at the tips of roots and of stems. The apical meristems[73] give rise to the primary meristematic tissues, protoderm, procambium, and ground meristem, eventually consisting of differentiated specific cell types in primary tissues[74]. This growth is termed primary growth, whereas secondary growth, which does not occur in monocot shoots, is derived from secondary or lateral meristems (*e.g.* vascular cambium).

Specimen 3: Prepared slide of longitudinal section of root apical meristem

1. Observe at 10x.

[70] Fig. 22-12
[71] Fig. 22-11
[72] Fig. 22-12
[73] Fig. 23-1
[74] Fig. 23-2

2. Observe the faint region at the lower tip of the root. This is the root cap[75], which protects the meristem from abrasion by soil.

3. Observe the region with small cells just above the root cap. This is the apical meristem, the region of cell division.

4. Observe the region where cells are more elongated than at the meristem. This is the region of cell elongation.

5. Observe the region above the region of elongation, where the cells in the rows appear different from on another. This is the region of cell maturation and differentiation. In this region, you may find root hairs.

6. Draw the longitudinal section of the root tip at 10x and label the root cap, apical meristem (region of cell division), region of elongation, and region of maturation. Include details of cell structure across a small portion of each region.

Specimen 4: Prepared slide of longitudinal section of shoot apical meristem

1. Observe at 10x.

2. Observe the domed tip of the stem. This is the apical meristem, the region of cell division. Notice that the cells are small and dense.

3. Observe the leaf primordia, which develop into leaves, arising from the perimeter of the apical meristem.

4. Observe the knob-like bud primordial at the base of the leaves. The bud primordia can develop into branches.

5. Draw the longitudinal section of the shoot tip at 10x and label the apical meristem, leaf primordia, and bud primordia.

Tissue systems. There are three tissue systems[76] that arise from the primary meristematic tissues. The dermal tissue system originates from the protoderm, the ground tissue system originates from the ground meristem, and the vascular tissue system originates from the procambium. Some tissues are composed of only one cell type and called simple tissues, whereas others, such as the epidermis, are composed of more than one cell type and are called complex tissue. Each of the major plant parts (roots, stems, and leaves (recall the floral organs are modified leaves)) is made up of these three basic tissue types. In each plant part, these tissues have specialized cells and thus perform specialized functions.

Dermal tissue. The dermal tissue[77], epidermis, is the outermost tissue layer (unless significant secondary growth occurs). Most of the epidermal cells are compactly arranged, providing mechanical protection and the walls of the epidermal cells of the aerial parts of the plant are covered with a cuticle, composed mainly of cutin and wax, to minimize water loss. Interspersed among the epidermal cells are pairs of specialized cells called guard cells[78]. Each pair of guard cells creates a pore, stoma (pl. stomata), between them. Through stomata, CO_2 for photosynthesis is taken up and

[75] p. 530, Fig. 24-4, Fig. 24-5
[76] Summary table pp. 526-527
[77] pp. 523-525
[78] Fig. 23-24

water vapor evaporates. The guard cells regulate the aperture of the pore. Trichomes[79] are another specialized type of dermal cell. Trichomes of roots, root hairs, facilitate the absorption of water and minerals from the soil. Trichomes of leaves reflect solar radiation to decrease leaf temperature and water loss, secrete defensive chemicals, deter insects from eating leaves, and, for carnivorous plants, trap insects.

Ground tissue. Ground tissue[80] usually forms the bulk of the tissues of a plant and is primarily involved in photosynthesis and storage. The cell types that comprise the ground tissue are parenchyma, sclerenchyma, and collenchyma. Parenchyma cells are living at maturity and usually have only a primary cell wall. Parenchyma cells are capable of cell division and therefore are important in regeneration and wound healing. Sclerenchyma cells, at maturity, lack protoplasts. Sclerenchyma (from Greek *skleros* meaning hard) cells have thick secondary cell walls and provide mechanical support. There are two primary types of sclerenchyma cells, fibers (*e.g.* of hemp, jute, and flax) that are long and slender and sclerids that are variable in shape. Sclerids are found in many seed coats, nutshells, and the stones of fruits like peaches. Collenchyma cells are living at maturity and are commonly found in strands beneath the epidermis in stems and petioles. Their unevenly thickened, nonlignified primary walls are soft, pliable, and supportive, especially for young tissues.

Vascular tissue. Vascular tissue[81] is specialized for transport and forms a continuous system throughout the body of the plant. The vascular system consists of two major tissue types, the xylem and the phloem, that are derived from the procambium region of the meristem.

Xylem is specialized for water and mineral transport and provides support. The water and mineral transporting cells of the xylem are the tracheary elements, which have secondary walls and maybe tertiary walls and lack protoplasts at maturity. There are two types of tracheary elements, tracheids and vessel elements. Tracheids, which lack perforations, are less specialized than vessel elements. Vessel elements are perforated and stacked end-to-end and are therefore efficient water-conducting cells. The xylem tissue may also contain fibers for support and parenchyma for storage.

Phloem transports many substances throughout the plant including sugars (produced from photosynthesis and transported to parts of the plant that are not photosynthetic), amino acids, plant growth regulators, proteins, and RNA. The transporting cells of the phloem are the sieve elements, which do not have secondary walls. There are two types of sieve elements, sieve cells (found in gymnosperms) and sieve-tube elements (found in angiosperms). Sieve-tube elements are stacked and are connected by sieve plates, which are large pores in the cell walls; thus, forming a tube. Associated with each sieve-tube element is one (or sometimes two) companion cell that maintains the enucleate sieve-tube element and loads and unloads substances to and from the sieve-tube element/companion cell complex.

Organization of tissue and cell types throughout the plant body. We will now study the arrangement and functional specialization of these tissue types in different plant parts. Note that we will study only primary tissues in this lab.

Specimen 5: Prepared slide of cross section through a dicot root (*Ranunculus*)

1. Observe at 4x. Observe the outer-most layer of cells, the epidermis, and the inner core, the vascular cylinder. The rest of the tissue is ground tissue.

[79] Fig. 23-27
[80] pp. 513-515
[81] pp. 516-522

2. Observe the ring of cells that separates the ground tissue from the vascular tissue. Zoom in on one of the cells of this ring of cells with the 10x and then the 40x objectives. This ring of cells is the endodermis and each cell is an endodermal cell. Notice that the side-walls and the outer walls are thicker. The walls of these cells perform a specialized function during water uptake by the root as will be discussed in more detail later.

3. Observe at 10x the layer of cells immediately interior to the endodermis. This layer is the pericycle. Cell division in the pericycle results in formation of lateral roots; thus, the pericycle is considered a meristem.

4. Observe at 10x the vascular cylinder. The xylem forms and x-shaped core and the phloem is between the "arms" of the xylem core in dicot roots.

5. Draw the root cross section at 10x and label the epidermis, ground tissue, vascular cylinder, endodermis, pericycle, xylem, and phloem.

Endodermis. Endodermal cells are specialized to accomplish the critical function of requiring the solutes that enters the root to cross a plasma membrane prior to transport by the xylem throughout the plant. The cell wall of each endodermal cell is banded by a Casparian strip. This strip is made of a fatty substance (suberin), which is impermeable to water. Water flows from the soil into the root through the cell walls of the epidermal cells and the cells of the ground tissue. The cell wall is non-selective, and all dissolved substances enter the root. But the water cannot cross the Casparian strip, and is therefore forced to enter the endodermal cell through the plasma membrane. The selective plasma membrane of the endodermal cell acts as a filter.

Specimen 6: Prepared slide of a cross section through a dicot stem (*Ranunculus*)

1. Observe at 4x. As in the root, the epidermal cells are on the perimeter of the cross section.

2. Observe the vascular bundles that form a ring within the cross section. The ring of vascular bundles is characteristic of dicots; whereas, vascular bundles of monocots are scattered throughout the stem. The ring arrangement of vascular bundles is important for the secondary growth that occurs in dicots, but does not occur in monocots. The region between the epidermis and the vascular bundles is composed of parenchyma cells and is called the cortex, part of the ground tissue. The region enclosed by the ring of vascular bundles is the central pith, also part of the ground tissue.

3. Observe one of the vascular bundles at 10x. Observe the large vessels of the xylem tissue closer to the pith and the phloem closer to the cortex. The cells between the xylem and phloem are the cambium, which is the progenitor of secondary growth.

4. Observe the cells that surround each bundle, they are the bundle sheath cells.

5. Draw the dicot stem cross section at 10x and label the epidermis, cortex, vascular bundles, pith, xylem, phloem, and cambium.

Specimen 7: Prepared slide of a cross section through a monocot stem (*Zea mays*)

1. Observe at 4x. Observe the arrangement of vascular bundles in the monocot.

2. Draw at 10x and label the epidermis, ground tissue, vascular bundles, phloem, and xylem.

3. In your notebook, write a comparison of the arrangement of vascular bundles of dicot and monocot stems.

Review questions

1. What are the primary functions of roots and what aspects of root structure facilitate each function?

2. During germination, from where does a seedling get nutrients to grow? After germination and throughout the life of the plant, from where does a seedling get nutrients to grow?

3. Draw a sketch, at the tissue level, of what a dicot stem would look like after significant secondary growth and label the regions of phloem, cambium, and xylem. How is the arrangement of vascular bundles conducive to secondary growth in dicots, but not in monocots?

Chapter 7

Regulation of Gas Exchange of Terrestrial Plants

Objectives

Leaves. Understand the relationship between structure and function of the cells and tissues of leaves.

Stomata. Know the function and regulation of stomata. Know the importance of water in agriculture and the rationale for studying guard cells. Understand how guard cells are expected to respond to some environmental conditions (*e.g.* CO_2 concentration, light, and drought).

Experimentation. Understand the principles and procedures of the experiment. Experience data collection and analysis.

Leaf structure[82]

As do roots and shoots[83], leaves consist of three major tissue systems: dermal tissue, ground tissue, and vascular tissue, which all arise from primary meristem activity. These systems are continuous throughout the plant body. Within leaves, sugars are produced by reduction of CO_2 during photosynthesis generally in parenchyma, which is part of the ground tissue system. Sugars produced from photosynthesis are transported (bulk flow mechanism) from leaves to sinks, which are heterotrophic (*e.g.* roots) by the phloem (vascular tissue). Phloem transport is driven by positive pressure created by osmotic influx of water. Water is transported throughout the plant body by the tracheary elements of the xylem (vascular tissue) via negative pressure driven by evaporation of water from the leaf. The water content in leaves is very high. The waxy cuticle excreted by the leaf epidermis prevents leaves from losing too much water; however, some water loss is necessary to bring nutrients to the leaves. In addition; however, the waxy cuticle prevents uptake of CO_2, into the leaf, which is necessary for photosynthesis. Thus, to allow gas (water vapor and CO_2) exchange in terrestrial plants, the leaf epidermis is perforated by adjustable pores, or stomata. Each stoma is flanked by a pair of guard cells, highly specialized epidermal cells, which are embedded in the tightly packed layer of epidermal cells. The pair of guard cells regulate the aperture of the stoma to balance water loss and CO_2 uptake. Guard cells balance water transport and photosynthesis; therefore, guard cells are regulated by many external (*e.g.* light) and internal (*e.g.* ABA synthesized by water-stressed roots and transport via xylem to guard cells) factors associated with both water status and photosynthesis. When the guard cells are stimulated (*e.g.* by light) to increase the aperture of the stoma between them, the first step to opening is activation of the H^+-ATPase in the plasma membrane of the guard cells. The energy from the hydrolysis of ATP drives the energetically uphill excretion of protons resulting in a more negative charge within the guard cells; thus creating a driving force for K^+ influx. The accumulation of K^+ causes osmotic water influx. Guard cells have specialized cell walls that are thick and rigid around the pore and radially wound cellulose microfibrils, so, as water osmotically enters guard cells, the cells bow out causing an increase in stomatal aperture. When stomatal closing is triggered (*e.g.* by ABA), anion channels are activated, allowing anions to passively move out of the guard cells, causing the charge in the guard cells to be less negative, resulting in efflux of K^+. K^+ and Cl^- efflux cause osmotic efflux of water that decreases the pressure in the guard cells thus decreasing the aperture of the stoma between them.

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[82] pp. 559-566

[83] Review Chapter 6

Specimen 1: Prepared slide of cross section of dicot leaf (*Ligustrum*)

1. Observe at 4x. Observe the upper and lower epidermis, the vascular bundles, and the remaining ground tissue. Notice that these are the same basic tissue systems that are found in roots and shoots.

2. Focus on the epidermis. Identify a stoma and observe at 10x. Each pore, stoma, is flanked by a pair of guard cells.

3. Observe at 40x the cells of the ground tissue, which in leaves is also called mesophyll (parenchyma cells of ground tissue). These cells are variable in shape and location. The closely packed columnar cells just below the upper epidermis are palisade parenchyma cells. Notice that each cell contains many chloroplasts. Below the rows of palisade parenchyma cells are the spongy parenchyma cells. Notice these cells also contain many chloroplasts. Also notice that there is much airspace between these cells. The organization of the leaf (air spaces near the stomata that allow CO_2 uptake and densely packed parenchyma with many chloroplasts that absorb light energy near the upper epidermis) is functionally efficient for photosynthesis.

4. Observe the major vascular bundle in the center that corresponds to the mid-vein of the leaf and the smaller vascular bundles that correspond to the minor veins of the leaf. Notice the orientation of the xylem and the phloem.

5. Draw the leaf cross section at 10x or 40x and label the upper epidermis, lower epidermis, guard cells, stomata, palisade parenchyma, spongy parenchyma, vascular bundle, xylem, and phloem.

Water is a limiting resource for plant growth

Fresh water is the limiting resource for terrestrial life. For perspective, about 85% of consumed water is by agriculture and, in the US, about 1,700 gallons of water are required to grow food for one adult per day[84]. Farmers strive to optimize growth with minimal water usage. Therefore, it is important that we understand how plants optimize water-use efficiency and, because guard cells regulate water loss, it is important that we understand guard-cell physiology. Experiments, as we are conducting in this laboratory, are vital for understanding biological systems.

Specimen 6: Epidermal peels of *Vicia faba* to observe guard cells.

1. Obtain a leaflet from a *Vicia faba* plant. Peel the lower epidermis back (as you did with the onion epidermis early in the semester).

2. Make a wet mount of the epidermal peel and observe at 4x and 10x.

3. Stain with a drop of Neutral Red for ~30 sec. Blot away most of the neutral red. Rinse the peel with a drop of tap water. Observe at 4x.

4. Draw at 40x and label guard cells, stoma, and epidermal cells.

5. While observing at 40x, count and record in your notebook the number of open versus closed stomata.

[84] National Geographic, 1993 Special Issue

Experimentation of guard-cell response to CO_2

Guard cells provide the major pathway for gas exchange in higher plants; they regulate CO_2 entry into, and water loss from leaves of higher plants. Thus, guard cells regulate the balance between photosynthesis and water conservation. We will use the model plant *Vicia faba* L. to determine how guard cells respond to atmospheric CO_2 concentration.

In your lab notebook, write the following.

1. The question that the experiment is designed to answer

2. Your hypothesis regarding how guard cells respond to atmospheric CO_2 concentration and rational for your hypothesis

3. A numbered procedure based on the procedural outline below

Outline of procedure (Your lab notebook should have more details of what you plan to do and what you do.)

Notice that at each node of the *Vicia faba* stem, there is a pair of leaflets on one petiole. Cut, under water to prevent an embolism in the xylem, one pair of leaflets including the petiole and one inch of the stem that is below the node and half of an inch of stem above the node. Quickly transfer into an Erlenmeyer flask containing water. Repeat this process for another pair of leaflets. One pair will be the control (atmospheric CO_2) and the other will be treated with low CO_2. Place the flask in a large beaker containing ~ 50ml of water or 0.5N NaOH[85]. Cover the beaker with Saran Wrap and leave under the light for 25 minutes. Peel the lower epidermis from leaves incubated under the two conditions. Stain the epidermal peel with neutral red for 1 minute then rinse it with tap water. Observe and measure at least 25 stomatal apertures from each sample with a microscope that has a micrometer in the ocular. Record the data in a table in your lab notebook. Mathematically analyze the results of the experiment and, independently, write an interpretation. Based on your data, what would you expect if you exposed droughted plants to low CO_2 conditions?

Write a short report as described in Chapter 1 of the lab manual. Note that all data analysis and writing must be done independently. Failure to compose lab reports independently will result in complete loss of credit for the report.

Review Questions

1. Why would non-functional guard cells result from symplastic connections via plasmodesmata between guard cells and other cells?

2. Assuming that the humidity inside leaves is 100%, under which atmospheric humidity, 90% or 40%, do you expect to see more open stomata? Why?

3. If you wanted to use a drug to inhibit stomatal closing, but not stomatal opening, what protein would you target the drug towards and why?

[85] NaOH depletes the concentration of CO_2 in the atmosphere.

Chapter 8

Data Analysis and Interpretation

Objectives

Statistics. Understand how and why statistics are used to analyze data. Understand calculations for the arithmetic mean and standard deviation of a set of values. Understand how and why the *t*-test is used for data analysis and interpretation.

Graphs. Know which type of graph (*e.g.* bar, line, or pie) is used for which type of data. Understand why graphs are useful for analyzing and interpreting data.

Introduction to Statistics

Government, scientists, doctors, lawyers, economists, businesses, and more utilize statistics. Health professionals may ask whether a medication is effective, for example, to lower blood pressure. To answer this question, experiments are performed on patients, and data, such as blood pressure measurements, are collected. Statistics and graphs allow the health professionals conducting the experiment to evaluate the effectiveness of the drug and to communicate their findings. Although health professionals use hundreds or thousands of patients to perform the experiment and would measure blood pressure many times throughout the experiment, producing thousands or hundreds of thousands of measurements, we will use an unrealistically small data set of ten patients and only the initial and final blood-pressure measurements for demonstration.

Basic analysis of Data

Arithmetic Mean. The arithmetic mean ($\bar{x}$) is the average. The arithmetic mean of a set of numbers is calculated by summing the numbers and dividing by the total number of values in the set.

$$\text{arithmetic mean } (\bar{x}) = \frac{\sum x_1}{n}$$

where x_1 represents each value and n represents the total number of values

For the example experiment designed to test if a medication is effective for lowering blood pressure (considering only diastolic[86]):

Initial diastolic blood pressure of patients who are given a placebo	Final diastolic blood pressure of patients who are given a placebo	Initial diastolic blood pressure of patients who are given experimental medication	Final diastolic blood pressure of patients who are given experimental medication
109	101	104	90
105	96	100	81
99	104	101	92
104	106	100	83
98	99	98	95
93	100	98	84
100	107	103	94
92	97	97	88
107	105	103	89
103	100	102	94

The arithmetic means for the four sets of measurements in the example are:

101	101.5	100.6	89

Standard Deviation

For the example, in which samples of the populations were measured, notice that the arithmetic means of the initial blood pressure measurements are the same, but the measurements varied more in the patients who received a placebo than those that received the medication. An indication of the variance around the arithmetic mean in a set of numbers better describes the set than the mean alone. Calculating the standard deviation around the arithmetic mean takes into account how much each value deviates from the mean of the values and the total number of values as shown in the following equation for standard deviation.

$$\text{standard deviation (s)} = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

Thus:

1. Subtract the arithmetic mean from each value and square the difference. (Note that squaring the difference eliminates the direction of the difference.)

2. Sum the differences from step 1.

3. Divide the sum of step 2 by the number of values in the number set minus 1.

4. Calculate the square root of the result of step 3.

The standard deviations for the four sets of measurements in the example are:

[86] Diastolic pressure is a measurement of the lowest pressure in the ventricles and atria when the heart relaxes after contraction in preparation for refilling during the cardiac cycle.

5.7	3.8	2.4	5.0

Thus, the variation around the arithmetic mean of the initial measurements of the patients given placebos was greater than that of the patients given medication. Variation is important for determining if differences in arithmetic means are indicative of differences in the set of values.

t-test

Based on the arithmetic means and the variance, do you think that the blood pressure medication affects diastolic blood pressure? Although the arithmetic mean final blood pressure for patients given medication is lower than that of patients given placebos, the variation in patients given medication is greater than those given placebos. Statistics provides an accepted method, the *t*-test, to determine if there is a difference between two sets of values. The *t*-test takes into account the averages and the standard deviations of the two sets of values being compared. The *t*-test results in acceptance or rejection of the null hypothesis that there is no difference between the two sets of values. The *t*-test is performed as follows mathematically; however, as described in the following section, software is an efficient way of performing the *t*-test.

1. Calculate the *t*-value from the following equation.

$$\frac{\left|\bar{x}_1 - \bar{x}_2\right|}{\sqrt{\left(\frac{{s_1}^2}{n_1}\right) + \left(\frac{{s_2}^2}{n_2}\right)}}$$

where subscripts 1 and 2 represent the two sets of values being compared

2. Calculate the degrees of freedom by $n_1 + n_2 - 2$ (*i.e.* n-1 for each set).

3. Use a *t*-distribution table (available on the internet or in any basic statistics textbook) to determine the critical *t*-value. To use the table, the degrees of freedom (above) and the critical *p*-value will need to be known. Determine the critical *p*-value that will be used to determine differences. For most biological experiments (and our purposes in this course), a *p*-value ≤ 0.05 (*i.e.* the probability that the two sets of data are different by chance is $\leq 5\%$) will be considered different. If the *t*-value for the comparison being made is greater than the critical *t*-value, one concludes a difference and *vice versa*.

In the example experiment, the *t*-value for the comparison of the initial and final blood pressures of the patients given placebos is 0.23 and for the patients given experimental medication is 6.75. Based on the *t*-distribution table, the critical *t* value is 2.1; therefore, there is not a difference between the initial and final blood pressures of those patients given the placebo, but there is a difference between the initial and final blood pressures of the patients given the experimental medication. From the *t*-test, we can conclude that the medication is effective at lowering diastolic blood pressure.

When the *p*-value is presented, as often given by software calculations (see below), significance is determined based on the critical *p*-value. If the critical *p*-value is 0.05, a *p*-value ≤ 0.05 (*i.e.* the probability that the two sets of data are different by chance is $\leq 5\%$) will be considered different. In the example experiment, the *p*-value for comparing the initial and final for patients who are given placebos is 0.82, and the *p*-value for comparing the initial and final for patients who are given medication is 0.000003. Thus, we would conclude that the medication has an effect on blood pressure, but the placebo does not. Note that the lower the *p*-value, the greater the difference is between the two sets of values (*i.e.* the greater the effect).

Although they are not described here, several important assumptions (*e.g.* random sampling) regarding the two sets of numbers that are being compared that must be considered when utilizing this test.

*Using software (*e.g. *Microsoft Excel) for basic statistics*[87]

Use of software to perform statistical analyses is accurate and efficient; however, it is important to understand the premises of the computations performed by the software and to be able to provide accurate information to the software regarding the experimental design and criteria. One common program that performs basic calculations and creates basic graphical representations is Microsoft Excel. To perform calculations such as those presented above the following procedure can be followed. In addition, utilize the Help menu.

Calculating the average and standard deviation with Microsoft Excel

1. Enter the data in table format (similar to the table in the above example).

2. Highlight the cell in which you want the result of the calculation to be displayed.

3. Go to the "Insert" menu and choose "Function."

4. To display all of the calculations that the program can perform, select "All" from the "Function category" in the left box.

5. To calculate, for example, the mean average, choose "AVERAGE" from the list.

6. A box will appear that requires the input information for the calculation. Ways to input the values for which you want to calculate the average follow. One, you can click the arrow to the right of the field and then select the cells in the spreadsheet that contain the values; by holding the mouse button and drag across all of the cells containing values to be included, you can select many cells at one time. (Then press enter.) Two, you can enter the numerical values into each number field. Three, you can enter individual cell coordinates into each number field.

7. Select "OK" and the result of the calculation appears in the selected cell.

8. Similarly, the standard deviation, "STDEV," can be calculated. (Follow the above, but substitute "STDEV" for "AVERAGE.")

Calculating the *p*-value with Microsoft Excel

1. Follow the first four steps above and choose "TTEST" from the available functions.

2. A box will appear that requires the input information for the calculation. See step six above for ways to input information from your table of values. (It is best to follow the first way). Remember the *t*-test is used to compare two sets of values (*e.g.* placebo and medication). For "Array 1," input the first set of values (*e.g.* placebo or control). For "Array 2," input the second set of values (*e.g.* medication or experimental). For tails, input "2" and for type, input "2."

3. The "TTEST" function results in the *p*-value. See the "*t*-test" section above for how to interpret the *p*-value.

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[87] a good website for instructions on using excel:
http://www.georgetown.edu/departments/psychology/researchmethods/computer/excel2.htm

Note: When calculating *t*-values and *p*-values using statistical programs, we will be performing two-tails and assume equal variance. Tails and types of *t*-tests are beyond the scope of this course; however, for more information about tails and types in these analyses, many resources are on the web or in a basic statistics textbook.

Graphs

Data are represented graphically in many different ways. The type of graph chosen to represent data depends on the type of data and the comparisons or relationships necessary for interpretation. In the example experiment involving blood-pressure medication, the data includes initial and final diastolic blood pressures for patients given either a placebo or the experimental medication; thus four averages and four standard deviations. To determine if the medication is effective a comparison between initial and final blood pressure would aid in interpreting the data. A line graph would not be appropriate because the blood pressure was not tracked throughout the experiment. A bar graph would be appropriate; each of the four averages (initial and final of placebo and initial and final of medication) will be represented by a bar. To create a bar graph in Microsoft Excel, the following procedure can be followed.

1. With the data in table format in Excel, choose "Chart" from the "Insert" menu.

2. Select the appropriate type of chart, in this case "Column," and select "Next."

3. Select "Series" at the top of the next box.

4. Select "Add" and name the series, *e.g.* "Initial Diastolic Blood Pressure." The series name can be entered by typing the name in the field or by using the arrow next to the field to select a cell of the spreadsheet containing the name of the series. Enter the values for the series by using the arrow next to the field and selecting the cell, or cells, that contain the numbers to be represented. (Use the control key to select multiple cells.) In the example, the values will be the arithmetic mean initial diastolic blood pressures.

5. Enter the x-axis labels by using the arrow next to the field to select the cells of the spreadsheet that correspond to the averages, *e.g.* Placebo and Medication.

6. If there is another series, *e.g.* Final Diastolic Blood Pressure in the example, follow steps 4 and 5 again.

7. When all the series have been entered, select "Next."

8. In the next box, enter titles for the graph and the axes and adjust any of the other parameter choices.

9. Select "Next" and then name the file, select the preferred location, and select "Finish."

10. Graphs can be formatted by double clicking on the elements (*e.g.* axes, background, bars, *etc*.).

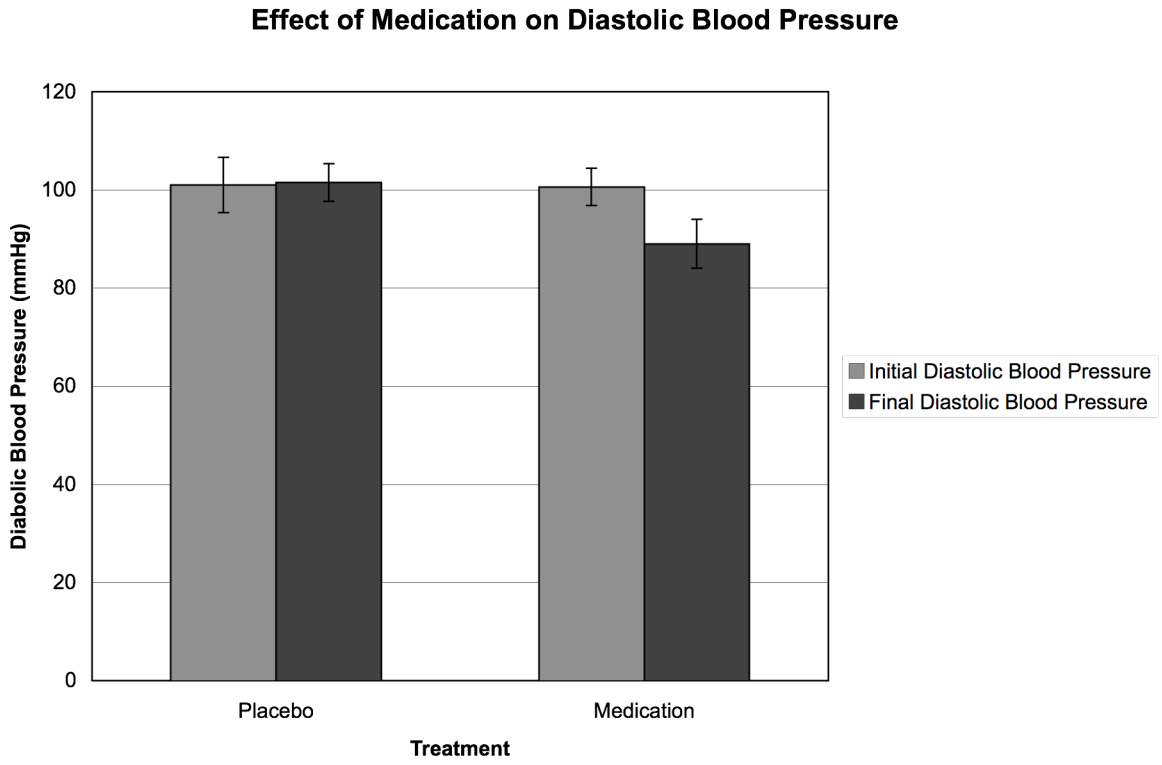
Now, the bars represent the arithmetic means, but it is important to represent the variation around the arithmetic means.

1. Right click on the bars and select "Format data series."

2. Select "Y Error Bars" from the top of the window.

3. Use the arrow next to "Custom +" to select the cells that correspond the standard deviation (the cells containing the standard deviations must be selected in the same order as the corresponding bars). Repeat with the "Custom – " field.

Finally, it is important to refer to the graphical representation and the statistical analyses of your data when discussing the experiment.



Review Questions

1. What type of graph would best represent the data from an experiment that was aimed to determine the effect of a plant-growth regulator on plant height if the height were measured every other day for 14 days? Without using numbers, sketch or use Excel to create the graph of measured control plants (without gibberellic acid) and experimental plants (with gibberellic acid). Include a graph title, axes titles, legend, data lines, and standard deviation bars.

2. If you needed to be treated for a condition, such as high blood pressure, with medication, would you choose a medication that, in studies, has a *p*-value of 0.05 and costs 2 cents per pill or a medication that has a *p*-value of 0.001 and costs 10 cents per pill? Why?

Chapter 9

Symbioses

Objectives

Symbioses. Understand the types of symbioses. Know examples of symbioses.

Mycorrhizae. Define the two major types of mycorrhizae. Understand how mycorrhizal associations in plants are beneficial to the associated plants and fungi. Know the abundance and specificity of mycorrhizal associations in plants. Know, in general, how mycorrhizae are visualized.

Symbiotic Associations

Coexistence of species has led to co-evolution and symbiosis. Symbiosis is a close association between two or more different species. Symbiotic associations are broadly categorized as parasitic, commensal, or mutual. Parasitism involves one organism that benefits and another that is harmed. Viruses, bacteria, fungi, animals, and even other plants may parasitize plants. Commensalism involves one organism that benefits from and another that is not affected by the association. One example of commensalism in plants is epiphytes, such as orchids and bromeliads, which grow on the stems and branches of high trees and benefit by gaining access to light, but do not harm or help the growth of the tree. Mutualism involves two species that both benefit from the association. Lichens are a mutualistic relationship between a fungus, which provides minerals and protection from dehydration, and a population of cells, algal or cyanobacterial, which provide fixed carbon and, if cyanobacterial, can provide fixed nitrogen. Lichens growing on tree trunks are a mutual symbiosis (fungus and algae or cyanobacteria) in a commensal symbiosis (tree and lichen).

Many plants are involved in an important mutual symbiosis with prokaryotes, the only organisms that can fix nitrogen ($N_2 \rightarrow NH_4^+$). The establishment of symbiosis between plant roots and nitrogen-fixing bacteria, commonly called rhizobia, is termed nodulation[88], in which tumor-like growths, nodules, form and consist of root cortical cells and bacteria. The plant provides energy and a low O_2 environment (O_2 inhibits nitrogen fixation by denaturing nitrogenase) and the bacterium provides fixed nitrogen, which is limiting second to water.

At least 80-90% of angiosperms, and all investigated gymnosperms are involved in a mutual symbiosis, termed mycorrhizae, with fungi. The plant provides carbohydrates and vitamins to the fungus and the fungus provides essential elements (especially phosphorous), protection against soil-dwelling pathogens, and an increased potential for water absorption[89]. Although many types of associations occur, there are two major types of mycorrhizal associations, endomycorrhizae and ectomycorrhizae. During endomycorrhizal associations[90], fungal hyphae evaginate against the plasma membrane, but do not enter the protoplast, of root cortical cells and form highly branched structures called arbuscules that increase in the surface area of contact between fungal and plant cells. Endomycorrhizal associations most often involve Glomales fungi (once an order of Zygomycetes), are not highly specific, and are more common that ectomycorrhizae. During ectomycorrhizal associations[91], fungal hyphae are usually found between epidermal and cortex cells

[88] Figs. 29-9, 29-10, 29-11
[89] Fig. 14-39
[90] Fig. 14-40
[91] Figs. 29-1, 14-41, 14-42, 14-43

and surround cells, but the plasma membrane does not invaginate. Ectomycorrhizal associations most often involve basidiomycetes and some are highly specific.

Investigating Mycorrhizal Associations

The goal is to observe, with staining, mycorrhizal associations and determine which types of associations are present in/around roots of selected species.

In your laboratory notebook, formulate a hypothesis based on your knowledge of mycorrhizal associations. Include rational for your hypothesis.

Protocol for staining mycorrhizae in roots

Caution: Wear proper laboratory clothing and gloves to protect your skin. Some chemicals in this protocol are hazardous.

1. Remove fresh root tissue from the specimen.
2. Rinse in tap water.
3. Transfer root tissue to a beaker containing 10mL (enough to cover roots) of 10% KOH.
4. Add 40µL of 30% H_2O_2 (hydrogen peroxide) and incubate for 10 minutes; however, if the solution turns to a yellowish brown color within the first 2-3 minutes, refresh the solution.
5. Transfer root tissue to a Petri dish containing tap water. Swirl the tissue in the tap water and let incubate for 5 minutes.
6. Transfer the roots to a Petri dish containing 10% HCl and incubate for 5 minutes.
7. Transfer the roots to a glass vial containing 0.05% aniline blue. Close the vial and place in water bath at 80°C for 30 minutes.
8. Transfer the roots to a Petri dish containing 85% lactic acid. Swirl gently and incubate for 10-15 minutes.
9. Make two wet mounts of the roots.
10. Observe and draw the results. Draw at least one wet mount and compare/contrast with the other wet mount specimen.
11. Describe the fungal structures (*e.g.* hyphae, arbuscules) in each of the roots observed.
12. In your lab notebook, answer the following questions.
    a. How are fungal hyphae distinguished from plant cells?
    b. What are your conclusions regarding the presence of mycorrhizae in the specimens observed?

Review Questions

1. How do you think fertilization affects mycorrhizal associations?
2. How are fungi ecologically important? Give at least two ways.
3. A plant-involving example of each type of the three major symbioses is presented in this chapter. Present an animal-involving example of each type of the three major symbioses.

Chapter 10

Overview of Autotrophic and Heterotrophic Protists

Objectives

Protista. Establish familiarity with the Protista. Understand some aspects of the importance of protists. Know the primary differences between autotrophs and heterotrophs. Define plankton and describe how they are important.

Autotrophic Protists. Know the taxa of autotrophic protists and the general characteristics used to determine these groups. Understand endosymbiosis. Understand the similarities and differences between zygotic meiosis and alternation of generations (a.k.a. sporic meiosis). Know the general morphological gamete forms and general types of sexual reproduction of protists. Know the similarities of and differences between green algae and plants. Become familiar with the diversity of green algae, and their structures, and life cycles. Understand the differences between and distinguishing characteristics of Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae, a photosynthetic heterokont).

Introduction to Protists

Protista comprises an assortment of primitive unicellular, colonial, and multicellular eukaryotes including simple photoautotrophic[92] organisms (*i.e.*. algae), protozoa (mobile, heterotrophic, and animal-like, *e.g.* Amoeba), and simple heterotrophic[93] organisms (*e.g.* slime molds and Oomycetes). This course will primarily focus on photoautotrophic protists. Three taxa of multicellular organisms, Plantae, Animalia, and Fungi, evolved from protists although protists do not have the distinguishing characteristics of any of the other kingdoms.

Protists are important in ecology and in studies of evolution. Photosynthetic protists in aquatic environments have similar importance as plants in terrestrial environments. Most protists are aquatic and are a major food source in aquatic habitats. Humans, in many cultures, eat protists, especially algae[94]. In addition, protists can cause disease and ecosystem disturbance. For example, when their habitat is disturbed, dinoflagellate populations explode, resulting in a "bloom" or in "red tide," that results in large quantities of toxic compounds accumulating and causing further disturbance of the ecosystem[95].

Protists are diverse and polyphyletic. They vary in size from one cell to tens of meters long (*e.g.* kelp, brown algae (Phaeophyta)). When present, motility of protists may be effected by flagella, cilia, or amoeboid movement. Protists may or may not have cell walls. The asexual and sexual reproductive cycles of protists are varied and include all three types of general life cycles; however, in general, protists lack complex reproductive structures. Three major types of gamete morpohologies in sexual reproduction[96] are observed in protists: isogamy (gametes are of equal size, shape, and motility), oogamy (one gamete is usually larger and is always nonmotile), and anisogamy (dissimilar gametes that do not meet the definition of oogamy). Anisogamy literally means "not the same" but it doesn't mean they both are flagellated, for example, although they are often portrayed this way.

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[92] photosynthetic and therefore do not require an external source of reduced carbon
[93] depend on external sources of reduced carbon.
[94] p. 300
[95] p. 302
[96] Fig. 15-15

Autotrophic Protists

Photosynthetic protists were classified historically on the basis of pigmentation, cell-wall composition, form and location of carbohydrate storage and other features. Although our understanding has been vastly improved by molecular analysis, the general groups are still valid. Four of the major taxa are Chlorophyta (green algae), Rhodophyta (red algae), Phaeophyta (brown algae), and Chrysophyta (diatoms). The word alga is not a formal taxonomic term and is often used to include cyanobacteria (or blue-green algae) even though cyanobacteria are prokaryotes. Cyanobacteria are the evolutionary progenitors of all plastids, through a single endosymbiotic event. Photosynthetic protists are extraordinarily diverse in sexual reproduction and all three of the general sexual reproduction methods, as well as complex variations, are presented.

Green Algae (Chlorophyta). Chlorophyta are themselves a diverse group. Plants evolved from a green alga. In general, green algae and plants contain chlorophylls *a* and *b*, store starch in plastids that have grana and two limiting membranes, and have cell walls made of cellulose; however green algae, in general, do not have complex reproductive structures as in plants and none are embryophytes. Green algae may be unicellular (*e.g. Chlamydomonas*), colonial (*e.g. Volvox*), filamentous (*e.g. Spirogyra*), "semi-filamentous" (*e.g.* desmids), or multicellular (*e.g. Chara* and *Nitella*). Green algae undergo asexual and sexual reproduction.

Specimen 1: *Chlamydomonas*

1. Place a drop of *Chlamydomonas* culture on a slide.
2. Add a drop of methyl cellulose to the culture, mix well, and add a coverslip. Methyl cellulose slows the movement of *Chlamydomonas*.
3. Observe under 40x. Observe the single large chloroplast in each cell.
4. Try to observe the orange "eye-spot" (stigma) that is involved in light sensing.
5. Dim the microscope light to observe the pair of anterior flagella that provide motility. Although flagella (~0.3μm) are below the resolution (~1μm) of the microscopes used in this course, they are visible by optical effects.
6. Describe the movement of *Chlamydomonas*.

Chlamydomonas reproduces asexually and sexually[97]. In our example, sexual reproduction is through zygotic meiosis; thus, except for the zygote, it is haploid. All cells—normal vegetative cells and cells that are gametes—are similar; therefore, it is is isogamous. Asexual reproduction by cell division occurs most frequently and sexual reproduction is induced by unfavorable conditions. When cells are exposed to unfavorable conditions, such as nitrogen deprivation, (+) or (-) haploid strains of cells produce, through mitosis, (+) or (-) haploid gametes respectively. Gametes then pair anteriorly, undergo plasmogamy, then karyogamy, resulting in a diploid zygote, the zygospore. The zygospore has a thick, protective wall that can withstand unfavorable conditions. When favorable conditions return, the diploid nucleus of the zygospore undergoes meiosis (zygotic meiosis) forming four haploid cells.

Specimen 2: Sexual reproduction of *Chlamydomonas*

1. Place a drop of (+) gametes on a slide.
2. Place a drop of (–) gametes a small distance from the (+) gametes on your slide. (Do not mix the pipettes for obtaining the two strains.)
3. Gently mix the drops containing the two types of gametes.

[97] Fig. 15-41

4. Create a slightly raised coverslip by doing the following. Wipe a thin layer of petroleum jelly on the back of your hand or on a paper towel. Gently, scrape two opposing edges of a coverslip through the thin layer of petroleum jelly.
5. Lower the coverslip, petroleum side down, onto a slide to create a small gap between the coverslip and slide. Then pipet the mixture of cells under the raised coverslip.
6. After ~3 minutes, observe at 40x and look for clumping.
7. After ~5 minutes, observe at 40x and look for pairing.
8. Draw *Chlamydomonas* clumping and pairing and label each cell and pair of flagella.

Specimen 3: *Volvox*, a colonial green alga

Volvox[98] is the dead-end evolutionary pinnacle of a colonial green alga that is based on *Chlamydomonas*-derived cells. The number of cells in the colony is specific to the particular species and ranges from 500-60,000. The cells are connected by cytoplasmic connections that allow cell-to-cell communication. The colonies are hollow and the cells on the periphery are biflagellate. The colony moves by synchronized beating of the flagella. Each cell has an eye-spot that detects light. Some cells are specialized for sexual reproduction through oogamous zygotic meiosis. Some cells are specialized for asexual reproduction and, thus, undergo mitosis to form small daughter colonies within the parent colony. When a daughter colony is mature, it breaks from the parent colony, increases in size by cell growth, and becomes free-living.

1. Prepare a raised coverslip as for specimen 3.
2. Place a drop of *Volvox* culture under the coverslip.
3. Gently add a coverslip.
4. Observe the spherical mobile colonies at 10x.
5. Look for daughter colonies.
6. Under dim light, try to observe cytoplasmic connections, flagella, and the eye-spots of the cells. (Although, these may be below the resolution of the microscopes).
7. Draw a colony of *Volvox* at 10x and label cells, daughter colonies, and flagella.

Specimen 4: *Spirogyra*, a filamentous green alga

1. Place a few filaments of *Spirogyra* on a slide and make a wet mount.
2. Observe under 10x. Observe the row of cells that comprises each filament.
3. Observe the spiral-shaped chloroplast(s) within each cell.
4. On each chloroplast, observe the pyrenoids, which were present, but not visible, in *Chlamydomonas* and *Volvox* chloroplasts also. The major functions of pyrenoids are to synthesize and store starch.
5. Observe the nucleus in each cell.
6. Draw and label the cells, cell walls, chloroplasts, pyrenoids, and nuclei of a filament of *Spirogyra* at 40x.

Spirogyra[99] undergoes asexual reproduction by cell division and fragmentation and also undergoes sexual reproduction by zygotic meiosis. During sexual reproduction, a conjugation tube forms between two filaments. Fertilization occurs either in the tube or after a gamete migrates through the tube into the other filament. The zygote becomes surrounded by a thick wall that enables it to survive in harsh conditions.

[98] Fig. 15-42
[99] Fig. 15-52

Specimen 5: Sexual reproduction of *Spirogyra*

1. Observe a prepared slide of *Spirogyra* under 10x.
2. Note the pair of filaments arranged side-to-side. Each filament is composed of haploid cells. One filament is of the (+) type and the other is of the (-) type.
3. Observe the conjugation tube that forms between two filaments. Following fusion, the cytoplasm of the cells combine allowing the genetic material to fuse to form a diploid zygote that will, when conditions are favorable, undergo meiosis.
4. Draw at least two stages of conjugation. Label gametes, conjugation tube, and zygote. Indicate haploid and diploid parts.

Specimen 6: Desmids, semi-filamentous green algae

Desmids[100] are found in abundance in peat bogs. Desmids have two sections or semi-cells that are joined by a narrow isthmus. Cell division and sexual reproduction are similar to the related *Spirogyra*.

1. Place a drop of the mixture of desmids on a slide and add a coverslip.
2. Observe under 10x. Several genera of desmids are represented.
3. Observe the two sections of each cell. Each semi-cell, or half-cell, contains a chloroplast and pyrenoid, but they share a single central nucleus.
4. Draw and label the half-cells, chloroplasts, and nucleus (or indicate where the nucleus would be found) of two different desmids at 40x.

Specimen 7: Complex multicellular green algae, *Chara*[101] and *Nitella*

As evolutionary precursors to plants, complex, multicellular green algae have features that are similar to plants. Some similarities, in addition to the similarities for all green algae, between complex, multicellular green algae and plants include presence of complex reproductive organs, node-like structures, and apical growth. However, the complex green algae are not embryophytes, a distinguishing characteristic of all plants.

1. Cut a small portion of the *Chara* "stem" and place on a drop of water on a slide. Notice that the body is brittle. This is caused by $CaCO_3$ (calcium carbonate) encrustation in the cell walls.
2. Observe the nodes, internodes, and branches.
3. Observe under 4x.
4. Try to find and focus on the red structures, which are complex reproductive structures termed gametangia, on the main filament or the branches. The gametangia make sperm or eggs, by mitosis, that are released into the surrounding water. External fertilization is followed by the formation of a diploid zygote that then undergoes meiosis.
5. Draw and label the main filament, branches, nodes, and gametangia of *Chara* without magnification and/or under 4x.

Preserved specimens of other interesting green algae

Observe the preserved specimens of *Acetabularia* (mermaid's wine glass) and *Valonia* ("sea grass"). These are marine green algae and are coenocytic, meaning it is multinucleate. Notice how large a single cell can be!

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[100] Fig. 15-53
[101] Fig. 15-56

Observe the preserved specimen of *Ulva lactuca* (sea lettuce). This marine green alga undergoes alternation of generation.

Red Algae (Rhodophyta)[102]**.** Unlike green algae, few red algae are unicellular. The chloroplasts of red algae closely resemble cyanobacteria. Chloroplasts of red algae contain abundant linear light-harvesting pigments, which mask chlorophyll *a* (red algae do not have chlorophyll *b*). These linear pigments give red algae their distinctive color and are well-suited for absorption of the wavelengths of light that penetrate deep waters where red algae are found in abundance. Red algae reproduce asexually by discharging spores and sexually by what can be exceedingly complex multiphase means.

Preserved specimens of red algae

Observe the coralline red algae, which are predominately.

Observe the dried edible sea-weeds, which are important ingredients of oriental cuisine.

Brown Algae (Phaeophyta)[103]**.** Brown algae are heterokonts, which have two unequal flagella at some stage in the life cycle. Brown algae undergo either alternation of generations[104] or gametic meiosis[105]. Brown algae range in size from microscopic to the largest of all sea-weeds, such as kelp and *Sargassum*; however, none are unicellular or colonial; all are complex. Brown algae have chlorophylls *a*, but not chlorophyll b. The light-harvesting pigment fucoxanthin gives brown algae their distinctive brown color.

Preserved specimens of brown algae

Observe the specimens of *Fucus*. Notice the blades and floats (air bladders), which keep the alga on the surface of the water.

Planktonic Protists

Planktonic organisms inhabit the water column of bodies of water and move with the currents.

Dinoflagellates[106]**.** Most dinoflagellates are unicellular biflagellates that live in marine and fresh-water habitats. Dinoflagellates have a characteristic complex, armor-like cell covering. Most dinoflagellates are autotrophic, others are heterotrophic or osmotrophic. Dinoflagellates undergo sexual and asexual reproduction.

Diatoms[107]**.** Diatoms are unicellular, colonial, or filamentous autotrophic organisms that live in marine and freshwater habitats. Diatoms are heterokonts, but typically lack flagella, except on gametes. Diatoms have characteristic walls made up of polymerized, opaline silica and consist of two overlapping halves.

Specimen 8: Planktonic Algae, Dinoflagellates and Diatoms

1. Observe a water sample under 10x and 40x.

[102] Figs. 15-29; 15-30
[103] Figs. 15-23; 15-24
[104] Fig. 15-27
[105] Fig. 15-28
[106] Fig. 15-5
[107] Fig. 15-20

2. Draw a few dinoflagellates and diatoms (at least two of each) that are present in the sample. Label each as a dinoflagellate or a diatom.

Heterotrophic Protista[108]

Oomycetes and Myxomycetes are examples of heterotrophic protists that may be free-living or parasitic. Superficially, these protists resemble fungi; however fungi have several distinctive characteristics. Aquatic and terrestrial forms of Oomycetes and Myxomycetes exist. The life cycle of Myxomycetes[109] is interesting because it has an animal-like amoeboid or plasmodial phase (no cell wall) and a fungus-like phase that produces spores. The plasmodial phase may grow into massive structures that span hundreds of square mile.

Review Questions

1. How are Chlorophyta similar to plants? How do Chlorophyta differ from plants?

2. Describe two ways that the life cycle of *Chlamydomonas* is different from the life cycle of plants.

3. Chlorophyta, Phaeophyta, and Rhodophyta all have chlorophyll *a*, what does that tell you about the photosynthesis in these organisms?

4. Describe endosymbiosis in the context of the theory that chloroplasts originated from cyanobacteria.

[108] pp. 309-312; 340-343
[109] Fig. 15-58

Chapter 11

Complex Heterotrophic Eukaryotes (Fungi)

Objectives

Kingdom Fungi. Know the characteristics of fungi. Know the ecological and economical importance of fungi. Understand the similarities and differences between fungi and other kingdoms of eukaryotes. Know some of the symbiotic relationships that involve fungi. Know the components of lichens and understand the roles of each component. Know the characteristics and life cycles, in detail, of zygomycetes, ascomycetes, and basidiomycetes.

Kingdom Fungi

The eukaryotic kingdom Fungi is diverse, versatile, and ecologically and economically important[110]. Fungi occupy diverse habitats (mostly terrestrial) and are important decomposers (saprobes) and participants in symbioses[111]. For example, the ascomycete *Sphacelia* can infect fescue grass (common cattle fodder); the fungus produces alkaloids that are poisonous to herbivores, thus protecting the grass. Fungi are ecologically important and essential; however, they can be an economical nuisance for food produces and distributors. Fungi can grow on living material; thus, causing diseases in plants and animals. Over 5000 species of fungi attack economically valuable crop and garden plants and over 175 species infect humans and domestic animals. For example, *Claviceps purpurea*, an ascomycete-type fungus, infects rye plants and produces alkaloid toxins that can be found in breads made with infected rye grains. These alkaloids, in high doses, cause the disease Ergotism (or St. Anthony's fire) that is accompanied by hallucinations, spasms, and convulsions; however in low doses, these alkaloids are used therapeutically to cause muscle constriction especially in treating high blood pressure.

Fungi are economically important. Certain yeasts produce ethanol and carbon dioxide and therefore are important for baking, brewing, and alcohol production. Fungi provide the distinctive flavors and aromas of some cheeses. Some fungi are delicacies, such as field mushrooms, shiitakes (both of which are grown locally), chanterelles (which are collected locally), truffles, and morels[112]. Importantly, fungi produce many antibiotics, such as penicillin. Cyclopsorin, derived from a soil-inhabiting fungus, suppresses the human immune reactions that cause rejection of organ transplants.

Characteristics of Fungi. Although they share some characteristics with plants and some with animals, fungi have a unique combination of characteristics[113]. All fungi are heterotrophic eukaryotes that are filamentous (filaments termed hyphae and mass of hyphae termed mycelium), unicellular, or coenocytic (rarely). All fungi have chitin-containing cell walls, in contrast to plant cell walls, which contain cellulose (although, a few fungi contain some cellulose in addition to chitin). Most fungi have no motile cells at any stage of their life cycle, in contrast to protists and most animals. All fungi reproduce[114] asexually and, sexual reproduction, when present, is by zygotic meiosis; thus, all cells except the zygote are haploid. The most common method of asexual reproduction is by spores, which are usually produced on either sporangia or conidigenous cells. Sexual reproduction in fungi consists of three phases: plasmogamy, karyogamy, and meiosis (to

[110] pp. 260-261
[111] pp. 285-291
[112] Fig. 14-3c
[113] pp. 262-265
[114] pp. 264-265

return to the haploid state). Thus, interestingly, as you will see in the laboratory exercises, some fungi have heterokaryotic cells (*i.e.* two or more have genetically different nuclei).

In this lab, we will examine three major groups[115] of fungi, Zygomyceta, Ascomyceta, and Basidiomyceta.

Zygomycetes[116]

A major ecological role of zygomycetes is the formation of mycorrhizae. During sexual reproduction of zygomycetes, they produce a zygospore, or zygosporangium, from which the name is derived. Hyphae of zygomycetes are coenocytic (aseptate) meaning the hyphae lack cross-walls and thus contain many nuclei within the same filament. The following describes the asexual and sexual reproduction of zygomycetes.

Specimen 1: Prepared slide of *Rhizopus* (bread mold)

1. Observe the hyphae of *Rhizopus* at 10x. Notice that the hyphae are aseptate and that the hypha is divided into two parts, the stolon (portion that traverses the surface of the substratum) and the rhizoids (portion that penetrates the substratum).
2. Observe the sporangia (a.k.a. sporangiophore, bodies for asexual reproduction) that produce the asexual spores at the tips of some hyphae. Asexual-spore-containing sporangia enlarge and separate from the rest of the hypha, forming a sac. Then, each nucleus in the sac, along with a bit of cytoplasm, is enveloped by a wall resulting in formation of a spore. When mature, the spores are released and germinate into individual hyphae.
3. During sexual reproduction[117], portions of two hyphae (now called gametangia) that are morphologically similar, but genetically distinct, form protrusions toward each other. The protrusions meet and the membranes fuse, forming a sac that then separates from the hyphae. The nuclei from the two hyphae fuse in pairs, and form a structure, the zygospore (or zygosporangium), containing many diploid nuclei. The zygospore[118] has a thick, protective wall that is resistant to harsh conditions. Under favorable conditions, the nuclei undergo meiosis. The zygospore then "germinates," giving rise to a sporangium with many haploid spores that then germinate to form new hyphae. Observe the fusing gametangia and the zygospores.
4. Draw, at 10x, and label hyphae, the stolon, rhizoids (if visible), sporangia, spores, gametangia, and zygospores.

Ascomycetes[119]

The ascomycetes include many familiar fungi such as truffles, morels, most yeasts, and some molds. During sexual reproduction, ascomycetes form a sac-like structure, the ascus, which bears sexual spores. Hyphae of ascomycetes are septate (cross-walls present); however, they are perforated, allowing organelles (including nuclei) and cytoplasm to pass through. Each hypha is made of many cells, and each cell contains one or two nuclei, depending of the stage of the life cycle. The following describes the asexual and sexual reproduction of ascomycetes.

Asexual reproduction of ascomycetes occurs by formation of uni- or multi-nucleate haploid spores, conidia[120], which form on stalks, conidiophores. Unlike zygomycetes, which produce spores within

[115] Table 14-1
[116] pp. 268-269
[117] Fig. 14-11
[118] Fig. 14-12
[119] pp. 269-272
[120] Fig. 14-15

a sporangium, ascomycetes produce spores externally. Sexual reproduction[121] of ascomycetes involves genetically different mating types (each of which undergo asexual reproduction also) that developed from different spores. When two compatible hyphae come in contact, large multinucleate sac-like gametangia form from each type of hyphae. Plasmogamy of the gametangia occurs producing dikaryotic (each cell has two haploid nuclei) hyphae. The dikaryotic hyphae proliferate by mitosis and organize into a fruiting body termed ascocarp. Dikaryotic cells lining the ascocarp grow into sacs called asci (pl.; ascus, s.)[122]. The two nuclei in each ascus fuse to form a diploid zygote (the only diploid cell in the life cycle) that undergoes meiosis to produce four haploid nuclei within the ascus. Each of the resultant four nuclei undergoes a mitotic division resulting in eight haploid nuclei. Each nucleus becomes surrounded by a thick wall, forming an ascospore (sexual spores vs. conidia, which are asexual spores). When the ascospores are mature, the asci rupture and release the spores, which germinate into haploid, monokaryotic hyphae.

Specimen 2: Prepared slide of a cross-section through the ascocarp of *Peziza*

1. Observe the ascocarp (entire structure) under 4x and 10x.
2. The ascocarp is lined by the asci, which each contain 8 (red) ascospores.
3. Draw, at 10x, and label the ascocarp, hyphae that form the ascocarp main structure, asci, and ascospores.

Specimen 3: Yeast (unicellular ascomycetes), *Saccharomyces cereviseae*

Yeasts[123] are economically invaluable for their use in cooking, brewing, and biotechnology. In addition, yeasts are important model organisms, especially for understanding molecular biology. Most yeasts are ascomycetes. Asexual reproduction of yeasts occurs by mitosis by budding (daughter cells of unequal size) or fission (daughter cells of equal size).

1. Place a drop of yeast culture (grown from commercial baker's yeast in a sucrose solution) on a slide.
2. Dilute with a drop of water
3. Observe at 40x and look for dividing cells.
4. Answer the following question in your lab notebook. Are these yeast undergoing budding or fission? What observation(s) led to that conclusion?

Basidiomycetes

Basidiomycetes[124] include mushrooms, puffballs, and some pathogenic fungi like the rust and smut fungi.

During sexual reproduction, basidiomycetes bear basidiospores (sexual spores) on the surface of a club-shaped basidium (s.; basidia, pl.) born on a basidiocarp. Hyphae of basidiomycetes have perforated septa; thus, each hypha is made of many cells and each cell contains one or two nuclei (number of nuclei depends on stage of life cycle).

Asexual reproduction in basidiomycetes is a variable character and less prominent than sexual reproduction. During sexual reproduction[125] of basidiomycetes, monokaryotic mycelia of different strains undergo plasmogamy forming a dikaryotic mycelium that forms the basidiocarp (the

[121] Fig. 14-14
[122] Fig. 14-16
[123] Fig. 14-31
[124] pp. 272-274
[125] Fig. 14-18

conspicuous mushroom) through mitosis. Basidia differentiate along the lining of the basidiocarp (*e.g.* the underside of the mushroom cap). The terminal dikaryotic cell undergoes karyogamy, which is immediately followed by meiosis. Each resulting haploid nucleus migrates into the tips of the basidia and is "cut off," forming basidiospores[126].

Specimen 4: Common commercial mushrooms, *Agaricus*

1. Obtain a young basidiocarp of *Agaricus*.
2. Observe the stalk and cap
3. Observe the veil around the cap and the annulus around the stalk. Both the veil and the annulus are derived from a protective covering over very young basidiocarps.
4. Slice the basidiocarp longitudinally.
5. Observe the gills on the underside of the cap. The gills bear the basidia (seen in the next specimen).
6. Draw and label the cap, stalk, gills, veil, and annulus.

Specimen 5: Prepared slide of a cross-section of the basidiocarp cap of *Coprinus*

1. Observe the slide under the 10x objective.
2. Observe the stalk and gills.
3. Observe the layer of basidia lining the gills.
4. Observe the basidiospores (red).
5. Draw, at 10x, and label the cap, stalk, gills, basidia, and basidiospores.

Smuts and Rusts. Smut[127] and rust[128] fungi are plant parasites that do not produce basidiocarps, but do produce basidia and basidiospores. Corn smut[129], *Ustilago maydis*, is a basidiomycete that parasitizes corn. The hyphae penetrate the tissue of the host plant and produce large distended growths in the corn kernels. At maturity, this growth is filled with the dikaryotic spores of the fungus that can overwinter. In the spring, karyogamy occurs in the spores, which then germinate to form a basidium and basidiospores, all within the corn kernel. The monkaryotic basidiospores germinate and the hyphae penetrate corn seedlings. A dikaroytic mycelium forms from plasmogamy of the monokaryotic hyphae and spreads through the corn plant causing formation of tumor-like growths.

Rusts have a complex life cycle, which usually involves the obligate, sequential infection of two different plant hosts. For example, the wheat stem rust[130], *Puccinia graminis*, infects barberry plants in the spring, and then form spores, which then infect wheat plants in the summer. An overwintering spore, released from infected wheat plants, then re-infects new barberry plants in the spring. The wheat rust is one of the most destructive of all the species of parasitic fungi. Some control has been achieved by eradicating barberry plants near wheat fields, but total eradication of the secondary host is probably not possible. In recent years, crop scientists have concentrated on developing rust-resistant wheat. This process is continuous because rusts adapt quickly to new varieties through mutation and natural selection.

[126] Fig. 14-19
[127] pp. 279-282
[128] pp. 278-279
[129] Fig. 14-30
[130] Fig. 14-29

Lichens

Lichens[131] are symbioses between a fungus (ascomycete or basidiomycete) and a green alga or cyanobacterium. The green alga or cyanobacterium supplies photosynthate to the fungus and obtains from the fungus amino acids, elements for growth, and physical protection from environmental extremes. Although the two components of lichens are clearly recognizable in the microscope, the external morphology of the lichen does not resemble either of its components. Lichens function differently from either the alga or the fungus alone. Lichens have a complex shape and structure that does not resemble either symbiont. Lichens are able to synthesize complex organic compounds, which neither the alga nor the fungus can produce. These compounds are often colored and some have an antibiotic effect.

It is still an open question whether the lichen association constitutes a case of mutually beneficial symbiosis or of a form of parasitism in which the photosynthetic alga is being utilized by the non-photosynthetic fungus. The latter assumption is supported by the following arguments: (1) In many (though not in all) lichens, fungal hyphae penetrate the algal cells and the algal cells may be killed in some lichen associations. (2) The fungus component reproduces sexually, while the reproduction of the alga is inhibited and is strictly asexual.

Lichens live on soil, trees, rocks, *etc*. They survive in extreme environments such as on high alpine rocks, deserts, *etc*. They are the principal components of vegetation in the treeless Arctic tundra (the so-called "reindeer moss" is a lichen) or in the Antarctic dry valleys where they live under the surface of rocks (endolithic forms). Lichens are particularly sensitive to air pollution and their disappearance is a biological indication of increased pollution level.

Review Questions

1. Describe at least one characteristic that fungi share with plants, but not animals, and one that fungi share with animals, but not plants.

2. What characteristics, or combination of characteristics, of fungi are not found in other kingdoms?

3. What are the distinguishing characteristics of zygomycetes, ascomycetes, and basidiomycetes?

4. How does the life cycle exhibited by fungi differ from that exhibited by plants?

5. How are fungi beneficial for farming? How are fungi detrimental for farming?

[131] pp. 286-290

Chapter 12

Biology of Non-Flowering Plants

Objectives

Overview of Non-Flowering Plants. Know the distinguishing characteristics of plants. Know the plant adaptations required for terrestrial life. Know the adaptations for terrestrial life displayed by angiosperms and how they are advantageous.

Bryophytes. Distinguish bryophytes from green algae[132] and from other plants. Understand the life cycle of *Marchantia*, a liverwort, and how it compares with the life cycle of higher plants *e.g.* gymnosperms and angiosperms. Know the structures of the bryophyte gametophyte and sporophyte and understand their function. Know the characteristics of peat moss.

Seedless Vascular Plants. Understand the advantages of a vascular system for terrestrial life. Understand the limitations associated with not having a seed. Understand how homosporous and heterosporous life cycles differ.

Gymnosperms. Know general features of gymnosperms. Understand the advantages of seeds and pollen for terrestrial life. Know the life cycle of gymnosperms. Understand how the structure of a gymnosperm leaf is adapted for terrestrial habitats.

Comparing Angiosperm and Gymnosperm Reproduction. Understand the similarities and differences in reproduction of angiosperms and gymnosperms. Understand the differences in seed formation in gymnosperms and angiosperms. Understand the advantages that each has over the other in terrestrial habitats.

Introduction to Non-flowering Plants

Several lines of evidence indicate that plants (embryophytes) evolved from green algae. Plants, unlike green algae, are, in general, terrestrial and have evolved adaptations for terrestrial life. Terrestrial plants require adaptations to avoid desiccation, provide mechanical support, transport water and nutrients, transfer "male" gametes, and protect the zygote from desiccation and harsh conditions. The three plant groups discussed in this chapter fulfill these requirements to various degrees. The basis for a comparative study of the groups of non-flowering plants is the evolution of adaptations for terrestrial life. Recall that angiosperms (flowering seed plants) are well-adapted for terrestrial life. Angiosperms have highly controlled stomata embedded in the cuticle-covered epidermis and stress-signaling hormones to avoid desiccation, complex vascular systems, efficient pollination mechanisms (characteristic flower), and seeds that are efficiently dispersed, protected, and nourished. In addition, comparing groups of plants, from bryophytes to angiosperms, notice the evolutionary advances. In addition to adaptation for terrestrial life, evolutionary advances in plants include sporophyte dominance over gametophyte dominance, the presence of seed, and oogamy over anisogamy or isogamy and non-flagellated over flagellated gametes.

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[132] p. 346

Bryophytes[133]

Bryophytes are the simplest plants, in which the gametophyte is the dominant, photosynthetic, and independent stage in the life cycle, which, recall, for all plants is alternation of generations. In bryophytes, the sporophyte is dependent, at least for a period, on the gametophyte. Bryophytes are not well adapted to terrestrial life because they have no (or rudimentary) vascular tissues, have no guard cells (although some bryophytes have rudimentary stomata), lack true roots or leaves, are seedless (recall a seed is composed of an embryo, nutritive tissue, and a seed coat), and, because they require external liquid water to complete their life cycle because they have flagellated sperm. Bryophytes are distinguishable as plants because the gametophytes of bryophytes have complex and multicellular reproductive organs, the antheridia (male) and archegonia ("female"); the archegonium protects the egg, zygote, and embryo, hence bryophytes are embryophytes[134] (distinguishing characteristic of all plants). Bryophytes are distinguishable from other plants by the sporophyte, which is unbranched (versus branched structures that are discussed with seedless vascular plants) and bears a single sporangium; thus is homosporous. Bryophytes are not considered evolutionary progenitors of other plants, but are considered an evolutionary branch. The three classes of organisms that constitute the bryophytes are the liverworts, hornworts, and mosses. The hornworts are the smallest group of bryophytes and are not discussed in this course.

Liverworts

Specimens 1 (macroscopic) and 2 and 3 (microscopic): Gametophytes of *Marchantia*, a liverwort

1. Observe the gametophyte of *Marchantia.* Like most liverworts, the gametophyte has a flat, simple structure (~10-30 cells thick) called a thallus[135] (s.; thalli, pl.).
2. Observe the underside of the thallus. Many thalli have rhizoids (but not true roots, which are defined by the presence of a vascular system) that anchor the plant to substrate. Some thalli have rudimentary stomata-like pores important for gas-exchange regulation, some have stomata similar to those found in higher plants, and some have no pores. The stomata-like pores are not regulated moment-to-moment as true stomata.
3. Observe the gemma cups. Gemma cups[136] are multicellular bodies that asexually give rise to new gametophytes.
4. Observe the reproductive structures[137] (gametangia), antheridiophores that bear "male" antheridia (flagellated sperm) and archegoniophores that bear "female" archegonia (non-flagellated and retained egg).
5. Draw the gametophyte and label the thallus, gemma cup, rhizoids, antheridiophore, and archegoniophore.
6. Next to each label, indicate the ploidy level of the structure.
7. Identify these structures in the *Marchantia* life cycle[138] sketch in your text.
8. Observe the prepared slide of the longitudinal section of the antheridiophore bearing antheridia (stained red) that contain flagellated sperm.
9. Draw and label the antheridia with sperm. Indicate ploidy level.
10. Observe the prepared slide of the longitudinal section of the archegoniophore bearing archegonia containing a single egg.
11. Draw and label an archegonium with egg. Indicate ploidy level.

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[133] Chapter 16, p. 345
[134] pp. 350-351
[135] Fig. 16-5
[136] Fig. 16-14
[137] Fig. 16-12 (macroscopic) and 16-7 (microscopic)
[138] Fig. 16-15 (Life cycle of a liverwort)

Specimen 4: Sporophyte of *Marchantia*, a liverwort

The sporophyte of *Marchantia* is formed after fertilization of the egg, within the archegonium, by the sperm. Following fertilization, the diploid zygote divides mitotically to form a diploid embryo[139], which grows further to form an adult sporophyte[140]. In *Marchantia*, the sporophyte is simple, microscopic, and dependent on the gametophyte for nutrition. The function of the sporophyte is to produce spores through meiosis.

1. Observe the prepared slide of the sporophyte, which is within the archegonia (of the "female" gametophyte).
2. Identify the sporophyte in the life cycle sketch of *Marchantia* in your text.
3. Observe the oval-shaped chamber, the "capsule," which contains the haploid spores (red), and is attached to the gametophyte by a stalk and a foot (at interphase of sporophyte and gametophyte). Sporophytes produce a single type of sporangium and, thus, release mature spores that are morphologically similar (making liverworts homosporous) that germinate to form a new gametophyte.
4. Draw and label the *Marchantia* sporophyte, capsule, spores, foot, and stalk. Also, indicate the ploidy level of the structures and the position of the gametophyte relative to the sporophyte.

Mosses. The mosses are the most complex group of bryophytes. They have a "leafy," erect gametophyte[141], which is more complex than that of liverworts. They do not have complex vascular systems, but have a rudimentary conducting system[142] with complex, multicellular rhizoids (root-like structures). In addition, many mosses have stomata[143] present on the sporophyte.

The antheridia and archegonia of liverworts are formed at the tips of the "male" and "female" gametophytes respectively[144]. Fertilization occurs within the archegonia and the zygote develops into the sporophyte by mitosis. The sporophyte is attached to and dependent on the gametophyte, even at maturity. The sporophyte of mosses is more conspicuous than that of liverworts. The sporophyte produces spores by meiosis and releases them by a dehiscence mechanism[145]. Mosses, like liverworts, are homosporous.

Peat Moss (*Sphagnum*) is common in wet places, predominantly in temperate and arctic regions. Its peculiar leaf-like structure[146], large, barrel-shaped, dead, colorless cells and a network of thick, living, photosynthetic cells between them, serves as a highly efficient water storage system.

Peat moss often forms extensive sphagnum bogs that can reach an age of 10,000 years or more. Because of the chemical activity of peat moss, the water stored in the bog is acidic, an environment that does not favor the activity of decomposing bacteria and fungi. Dead *Sphagnum* and other plants, including wind-carried pollen grains from other plants, accumulate in the deeper layers of bogs. The examination of cores from peat bogs can yield important information. For example, pollen-grain analysis in peat bogs reveals the composition and history of the surrounding vegetation because plants can be identified by their pollen grains, thus revealing the history of changes in climate (paleoclimatology).

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[139] Fig. 16-8
[140] Fig. 16-9
[141] Fig. 16-21
[142] Fig. 16-24
[143] Fig. 16-28
[144] Fig. 16-25 (Life cycle of a moss)
[145] Fig. 16-20
[146] Fig. 16-20b

Many remarkable archaeological finds have also been recovered from peat bogs. In 1986, human remains from thousands of years ago were discovered in a peat bog in central Florida. The remains were well-enough preserved to permit analysis of DNA recovered from the body tissues.

Seedless Vascular Plants[147]

A vascular system[148] and support mechanisms allow plants to attain size and complexity in terrestrial habitats. In addition, most seedless vascular plants have a cuticle covering the epidermis that prevents desiccation and stomata that regulate gas exchange. These structures evolved in the diploid sporophyte. In vascular plants, the sporophyte is the dominant, photosynthetic, macroscopic generation that is only dependent on the gametophyte only for initial development. However, free-living (exposed to the environment) gametophytes and flagellated sperm, which need external liquid water to move to the egg, are hindrances to terrestrial life for these plants.

Specimen 5: *Psilotum* (whisk fern), homosporous seedless vascular plant

1. Observe the sporophyte of *Psilotum*[149], which has a green, dichotomously (equally) branched stem that is photosynthetic and has vascular tissue. (Unequal branching leads to more complex structures, thus dichotomous branching is a simple trait.)
2. Observe, on the stem, the presence of small leaf-like structures call enations. Enations do not have vascular tissue within them.
3. Observe the sporangia near the tips of the branches. Sporangia make spores and release them when they are mature. Released spores germinate to form a free-living gametophyte, which makes antheridia and archegonia. *Psilotum* is homosporous; thus, each spore forms a bisexual gametophyte.
4. Draw the stem, showing dichotomous branching. Label the stem, enations, and sporangia. Indicate the ploidy level of each structure.

Specimen 6: *Selaginella*, heterosporous seedless vascular plant

Selaginella[150] is sometimes called a "resurrection plant," because, after severe desiccation, it is one of the few plants that can be restored. *Selaginella* has a bushy appearance with true leaves borne on a true stem and true roots. The tip of the sporophyte bears strobili (pl.; strobilus, s.). Strobili are cone-like structures that bear the spore-producing parts of the plant.

1. Observe the prepared slide of the whole-mount of the strobilus of *Selaginella*. The leaves on the strobilus are called sporophylls.
2. At the base of each sporophyll is a sporangium, which bears spores. There are two types of sporangia, the megasporangium bears megaspores (large spores) and the microsporangium bears microspores (small spores). Megaspores and microspores are released and germinate to form the "female" and "male" gametophytes respectively. The flagellated sperm produced in the antheridia of the "male" gametophyte fertilize the eggs produced in the archegonia of the "female" gametophytes, thus returning to the diploid generation.
3. Draw and label the micro- and megasporophylls, micro- and megasporangia, and micro- and megaspores. Indicate the ploidy level of each labeled structure.

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[147] Chapter 17 p. 368
[148] Fig. 17-3
[149] Fig. 17-34 (Life cycle of *Psilotum*)
[150] Fig. 17-17

Although most seedless vascular plants are homosporous, *Selaginella* is hetersporous. Heterospory is an evolutionary advancement in vascular plants and is the precursor to the production of seeds, which form the basis for the success of higher plants.

Specimen 7: Fern sori

1. Observe the underside of the leaf-like sporophyll of a true fern[151]. The brown structures are sori[152] (pl.; sorus, s.), which are specialized structures bearing clusters of sporangia.
2. Draw the underside of a fern sporophyll with sori.
3. Scrape a small amount of the brown "powder" onto a slide (not a wet mount). Observe and note the sporangia (elliptical sacs with thick-walled cells) that contain haploid spores.
4. Heat the slide gently. Observe the sporangia again under the microscope and notice that the spores have been released. The heating causes the thick-walled cells that line the wall of the sporangium to lose water, shrink, and cause the sporangia to release the spores. Ferns are homosporous; thus, each spore forms a bisexual gametophyte that is free-living and photosynthetic. Fertilization occurs within the archegonia, where the new sporophyte begins development.
5. Draw and label sporangia, spores, and the thick-walled cells lining the sporangia. Indicate the ploidy level of each labeled structure.

Gymnosperms

The four groups of living gymnosperms are cycads, ginkgos, gnetophytes, and conifers. As in ferns, the sporophyte dominates the gymnosperm life cycle; however, all gymnosperms are obligatorily heterosporous because they produce seeds. Gymnosperms have two structures that make them better adapted to a terrestrial environment than the seedless plants, seeds and pollen. In addition, gymnosperms have well-developed vasculature, including a vascular cambium that produces secondary growth.

The mature sporophyte (the familiar tree) of gymnosperms[153] produces microspores and megaspores that are born in sporangia of separate, morphologically distinct cones. Each scale of a "male" cone bears two microsporangia, which contain numerous microspore mother cells that, through meiosis, give rise to haploid microspores that each develop into a four-celled pollen grain. Each scale of a "female" cone bears two ovules, each containing a megasporangium that is surrounded by an integument. Each ovule contains a single megaspore mother cell that undergoes meiosis. Three of the four megaspores produced from meiosis of the megaspore mother cell disintegrate and the remaining megaspore forms into the female gametophyte, which is never shed by the sporophyte (another important attribute required for seed production). During pollination, two sperm nuclei enter the egg cell, but one disintegrates and the other unites with the egg-cell nucleus to form the zygote. The ovule develops into a seed, which is covered by a seed coat. The seed provides protection from unfavorable environments for the embryo and provides nutrients for early development of the new sporophyte. The development of seeds and pollen freed plants from the requirement of liquid water for reproduction.

Cycads[154]**.** Many cycads grow to heights of small trees and are frequently mistaken for palms because their unbranched stems are topped with a crown of palm-like leaves. *Cycas* is the most primitive living cycad. The pollen grains, which contain the sperm, are deposited on the ovule.

[151] Fig. 17-30
[152] Fig. 17-27; 17-28
[153] Fig. 18-17
[154] Fig. 18-33

Upon reaching the fluid-filled chamber above the female gametophyte, their pollen tubes burst and release flagellated sperm, which swim the rest of the way to the egg.

Specimen 8: *Cycas* megasporophyll

1. Observe the megasporophyll of *Cycas*, noting the position of the ovule.
2. Observe a preserved seed and note the presence of the female gametophyte at the center.
3. Outside the gametophyte is a distincy layer called the nucellus (remains of the megasporangium).
4. Outside the nucellus is the integument that will form the seed coat.
5. Draw and label the megasporophyll and ovule. Draw a section of the ovule and label megasporangia and female gametophyte. Indicate the ploidy level of each labeled structure.

Ginkos[155]**.** Like the cycads, the ginkgos were once distributed worldwide and formed extensive forests. Now, they are represented by a single species, *Ginkgo biloba*, which is native only to southeastern China. *G. biloba* is a highly branched tree with fan-shaped leaves, which are deciduous (shed each fall), but attractive ornamental plants in the United States.

Conifers. The dominant and most conspicuous gymnosperms are the conifers, which include the pines, spruces, firs, cedars, yews, junipers, and redwoods. Conifers are woody, perennial plants, either trees or shrubs. Most conifers are bisexual, meaning each individual produces both male and female cones. The leaves of conifers are either scale-like or needle-like in shape. Most conifers are also evergreen (retain each leaf for several years).

Specimen 9: Cross-section through a pine needle

Conifers tend to be drought-tolerant plants. The small leaves of conifers have a thick epidermis and cuticle and relatively few stomata. The stomata of pine needles are sunken in the epidermis, thus decreasing the driving force for water loss.

1. Observe the cross-section of a pine needle[156] and notice the thickness of the cuticle.
2. Look for sunken stomata.
3. At the center of the needle is a patch of vascular tissue (with tracheids and sieve cells), which is surrounded by photosynthetic cells.
4. The two large holes in the photosynthetic part of the leaf are resin ducts. Resin ducts are also found in the stems and roots of many conifers. The cells surrounding these ducts produce terpenes and resinous compounds that protect the plant against insect and fungal attacks.
5. Draw and label the epidermis, stomata, resin ducts, tracheids, and photosynthetic cells of the pine needle.

Specimen 10: Longitudinal section of the "male" cone (microsporangium) of pine[157]

1. Note the microsporophylls, scales, which bear the microsporangia[158].
2. Observe, within the microsporangia, the presence of several winged pollen grains[159]. Each mature pollen grain is a haploid male gametophyte.
3. Draw and label microsporophylls, microsporangia, and pollen grains.

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[155] Fig. 18-35
[156] Fig. 18-12
[157] Fig. 18-15
[158] Fig. 18-17
[159] Fig. 18-16

Comparison of Angiosperm and Gymnosperm Reproduction

The angiosperms, or flowering plants, are the most advanced, diverse, and numerous group of plants. Several major changes in reproduction occurred as the angiosperms evolved, making them more successful on land than other groups of plants. As in all vascular plants, the diploid sporophyte of angiosperms and gymnosperms is dominant in the life cycle of flowering plants. Reproduction by the sporophyte involves the production of microspores and megaspores by meiosis.

In angiosperms the microspores are produced in the anther (collection of microsporangia), which, in turn, is part of the stamen, which may be thought of as the fusion product of microsporophylls. The microspores develop into male gametophytes, or pollen grains. The angiosperm pollen grain is similar to that of the gymnosperms; however, pollination in most angiosperms is aided by animals rather than by wind. This proved to be an extremely important adaptive advance.

The female reproductive structures of both angiosperms and gymnosperms are enclosed in a structure called the ovule. In gymnosperms, the ovule is borne on the megasporophyll. In angiosperms, the ovule is borne on the carpel (megasporophyll) and the carpel(s) form an ovary in which the ovule(s) are enclosed. In the ovule, a single haploid megaspore is formed by meiosis. The angiosperm megaspore develops into a haploid female gametophyte, which consists of only 7 cells (containing a total of 8 nuclei).

After fertilization in gymnosperms the nutritive cell layer in the seed is the remnant of the female gametophyte. In angiosperms, the seed produces a triploid nutritive tissue (the endosperm) and the ovary wall develops into the flesh of the fruit.

Review Questions

1. Compare and contrast the alternation of generations that occurs in bryophytes and in gymnosperms. Give at least two similarities and two differences.

2. Contrast the fate of the spores in *Selaginella* with the fate of the spores in angiosperms.

3. Describe the evolutionary and adaptive advances displayed by each of the following groups of plants: ferns, gymnosperms, and angiosperms.